Genetic Studies on Cystic Fibrosis in Hawaii

S. W. WRIGHT AND N. E. MORTON[1]

Cystic fibrosis (CF) of the pancreas is an autosomal recessive disorder with onset in the newborn period, infancy, or early childhood (Lobeck, 1966). Clinical signs are associated with meconium ileus and intestinal obstruction in the newborn or with chronic pulmonary disease, steatorrhea, malnutrition, and growth failure in the infant and older child. Although it is suspected to be a disorder of exocrine gland function, the molecular basis for the disease is not known. Mortality is high; 50% or more of affected patients succumb by 15 years of age. Typical pathological features include fibrotic and cystic changes in the pancreas together with chronic pulmonary disease. The diagnosis is based on clinical signs associated with laboratory findings of increased sweat sodium and chloride excretion, absent trypsin activity in pancreatic secretions, or typical pancreatic changes at autopsy. In the newborn with meconium ileus, characteristic changes in the intestinal glands may be present with only minimal pancreatic involvement (Thomaides and Arey, 1963).

Reliable estimates indicate that the incidence of CF in the Caucasian population is between one per 2,000 and one per 4,000 births, with a corresponding gene frequency of .022 and .016, respectively (Steinberg and Brown, 1960; Kramm *et al.*, 1962; Merritt *et al.*, 1962; Danks *et al.*, 1965). The maintenance of a genetically lethal trait at this high frequency is more likely to be associated with some unknown biological advantage to the heterozygote rather than a high mutation rate or multiple loci. Efforts to detect this advantage have yielded negative or equivocal results (Danks *et al.*, 1965; Hallett *et al.*, 1965; Knudson *et al.*, 1967).

The incidence of CF in non-Caucasian populations is not known. The disease has been reported sporadically in American and African Negroes (Kulczycki *et al.*, 1964), Japanese (Yamamoto and Abe, 1957; Hamamoto *et al.*, 1961; Ikai *et al.*, 1965; Ikai, personal communication), and American Indians (Harris and Riley, in press). Either the disorder is rare in non-Caucasians and maintained only by recurrent mutation pressure or there is failure to recognize the disorder in these racial groups. There are isolated reports of the disease in non-European Caucasians, including Arab and Sephardic Jews (Levin, 1963), Indians (Kandar, 1961), and Lebanese (Salem and Idresh, 1962).

The present study was designed to determine the incidence of CF in the Cau-

Received August 30, 1967.

Supported by a grant from the Cystic Fibrosis Research Foundation. The computer analysis was carried out using the CDC 3100 supported by U.S. Public Health Service research grant FR 00247 from the National Institutes of Health. Dr. Wright is the recipient of a grant from the Commonwealth Fund.

[1] Department of Genetics, University of Hawaii, Honolulu, Hawaii 96822.

casian and non-Caucasian populations of Hawaii. The data necessary for this analysis were available from 24 affected patients with 64 ascertainments as well as from information on mating type frequencies for all live births from 1950 through 1965 (Morton *et al.*, 1967). The analysis was carried out using the SEGRAN program for estimation of incidence from the distribution of numbers of ascertainments in families with a probands among r affected (Morton, 1962).

METHODS

Period of Study

The study comprised data on all legitimate live births in Hawaii from January 1, 1950, through December 31, 1965.

Racial Classification

The complexity of racial and mating patterns in Hawaii has been reviewed by Morton *et al.* (1967). Seven major racial groups are recognized, comprising Caucasians, Hawaiians, Japanese, Chinese, Filipinos, Puerto Ricans, and Koreans. The Samoans, Negroes, Indians, Guamanians, other Pacific Islanders, and triracial groups other than part Hawaiians constitute a small group of "others." A final group includes "part Hawaiians not otherwise specified." This classification, including 21 biracial mating groups, gives 30 possible racial groups for each parent, or 900 potential mating types, of which 524 have been recorded in a previous study (Morton *et al.*, 1967). Racial classification is based on parental race as recorded on the child's birth certificate. Errors in classification are about 6% for Hawaiians and 1% or less for Caucasians, Japanese, Chinese, Filipinos, and Koreans.

The complexity and number of mating types, as well as the small number of patients with CF that were found in the non-Caucasian population, suggested that arrangement of the mating patterns to express the proportion of Caucasian ancestry in each parent would be most meaningful. Thus, the five Pacific races—Hawaiian, Japanese, Chinese, Filipino, and Korean—are grouped as non-Caucasian, and their proportion of Caucasian ancestry is classified as "0." These racial groups in Hawaii are represented approximately as follows: Japanese, 31%; Filipino, 11%; Chinese, 9%; Korean, 1%; and Hawaiian, 10%. Pure Caucasians, including Portuguese and Puerto Ricans, are classified as "1." The term "1/4 Caucasian" refers to a triracial parent in whom the ancestry is Hawaiian, Caucasian, and one other non-Caucasian race; previous studies have shown that these parents are approximately one-fourth Caucasian (Morton *et al.*, 1967). Illegitimate births, as well as births included under "others," have been omitted from the tabulations.

Hospitals

Autopsy and medical records were reviewed from the 11 largest hospitals in Hawaii: Tripler General, Queen's, Kauikeolani Children's, Kaiser, Kuakini, St. Francis, Kapiolani, Wilcox Memorial, Kauai Veteran's, Maui Memorial, and Hilo Memorial. These hospitals accounted for approximately 90% of the total live births during the period of study. Each hospital has a record librarian and a full-time pathologist. Diagnoses are coded according to the Standard Disease Classification system and, more recently, according to the International Classification system.

Tripler General Hospital is the major hospital for the U.S. armed services in the Pacific area; care of dependents is a major responsibility. It was recognized that the families of some affected infants born at Tripler General Hospital would have been transferred from Hawaii before the diagnosis could be established. Omission of these families would result in an underestimate of the incidence. To correct for this discrepancy, children born outside of Hawaii, but on whom the diagnosis was made after residence was established in Hawaii, have been included in calculation of the incidence.

TABLE 1

SOURCES OF 64 ASCERTAINMENTS ON 24 PATIENTS WITH CF: HAWAII, 1950–65

Autopsies	10
Hospital records	20
Physicians	15
Death certificates	7
Cystic Fibrosis Foundation	12
Total	64

NUMBERS OF 64 SEPARATE ASCERTAINMENTS ON 24 PATIENTS WITH CF: HAWAII 1950–65

Number of Ascertainments	Number of Patients
1	6
2	4
3	9
4	3
5	2
Total	24

Diagnoses

An accepted diagnosis of CF was based on the clinical history and one or more of the following criteria: (1) autopsy findings, (2) absent duodenal trypsin, and (3) elevated sweat sodium or chloride on two or more examinations.

Ascertainments

Ascertainment of affected patients was through five sources; these and the numbers of ascertainments are given in Table 1. Ascertainment was made in the following order:

1. *Autopsies.* Among the 11 hospitals, protocols were reviewed from 4,257 pediatric autopsies from birth through 15 years of age for the period 1950–65. The over-all autopsy rate for fetal, neonatal, infant, and child deaths was about 80%. Particular attention was given to those patients whose protocol indicated recurrent pulmonary disease, intestinal obstruction, meconium ileus, meconium peritonitis, malnutrition, or chronic intestinal disease. Autopsy material for these patients as well as those with CF was restudied. In those instances in which the microscopic findings were uncertain, the slides were reviewed by James B. Arey, pathologist for St. Christopher's Hospital, Philadelphia, for a final decision.

2. *Medical records.* During the period of this study, there were approximately 110,600 pediatric admissions to the 10 hospitals under study. Medical record librarians provided charts on patients with the following diagnoses: cystic fibrosis of pancreas, fibrocystic disease of the pancreas, mucoviscidosis, meconium ileus, meconium peritonitis, intestinal atresia, rectal prolapse, bronchiectasis, celiac syndrome, and malnutrition. When indicated, a follow-up was made to confirm or discard the diagnosis of CF. A tendency toward overdiagnosis was noted, and a number of Caucasian and non-Caucasian patients were shown by subsequent history, laboratory studies, or pathologic findings to have other disorders.

Ascertainments through CF clinics at Tripler General Hospital and Kauikeolani Children's Hospital are included under Medical Records.

3. *Death certificates.* The death certificate file maintained by the Hawaii State Department of Health was searched for additional patients with a diagnosis of cystic fibrosis.

TABLE 2

DISTRIBUTION OF 1,820 PEDIATRIC AUTOPSIES IN HAWAII, 1950–65, BY RACIAL CLASS AND FREQUENCY OF CF

Racial Class	Total	%	Number with CF	%
Caucasian	654	36	14[a]	2.14
Part Caucasian	382	21	0	0.0
Non-Caucasian	784	43	2	0.255
Total	1,820	100	16	.....

[a] Six of these patients are excluded from subsequent tabulations on incidence since they were born prior to 1950 or the initial diagnosis had been made outside Hawaii.

4. *Physicians.* All pediatricians in Hawaii and a number of general practitioners were contacted regarding patients under their care. Three children who had not been hospitalized for diagnostic study were ascertained in this manner.

5. *Cystic Fibrosis Foundation.* The local chapter was organized in 1962. A file is maintained of all families with affected children who have belonged to, or are known to, the organization.

RESULTS

CF in Autopsy Material: Hawaii, 1950–65

Information concerning the completeness with which Caucasian and non-Caucasian children are studied for evidence of CF was obtained from autopsy reports. Protocols were reviewed on 4,257 fetuses, infants, and children up to 16 years of age who died within the years 1950–65. To simplify the tabulations, 2,437 deaths on the following categories have been excluded: (1) stillbirths; (2) premature infants and newborns dying within the first three days of life, with a primary diagnosis of prematurity, congenital atelectasis, respiratory distress syndrome, or intracranial hemorrhage; and (3) 24 children for whom the race was unknown. The protocols on these patients were reviewed; none had cystic fibrosis.

Data on the remaining 1,820 pediatric deaths are given in Table 2. Patients were grouped into three broad classes, corresponding to the degree of Caucasian ancestry: (1) pure Caucasian, (2) part Caucasian, and (3) non-Caucasian. As shown in Table 2, 36% of the 1,820 autopsies were performed on Caucasian children and 43% on non-Caucasians.

Table 2 also gives information on the frequency of CF in the autopsy material. CF was present in 2.14% (about one in 50) autopsies on Caucasian children, as compared to only 0.255 (about one in 400) in the non-Caucasian group. This eightfold difference strongly suggests a significant difference in the frequency of the disorder between Caucasian and non-Caucasian races. A primary diagnosis of acute or chronic pulmonary disease not associated with CF was made in 12.4% of Caucasian, 21.5% of part Caucasian, and 16.6% of non-Caucasian infants and children.

Brief mention will be made of the two non-Caucasian patients with cystic fibrosis:

Patient No. 1, a female, was born in 1952. The father is Chinese, the mother Japanese. The paternal and maternal grandparents are from China and Japan, respectively. There is no consanguinity. There is one normal sib. The birth weight was 6 pounds, 3 ounces. The baby was operated on the fourth day of life for ileal atresia, and a side-to-side anastomosis was performed. She died two days postoperatively. At autopsy, two large meconium plugs were obstructing the anastomosis. Sections from the bowel showed a large accumulation of acidophilic secretions in the lumens of the glands. The lumens of the intestinal glands were dilated, the lumen cells flattened, and there was retention of secretions. These changes were compatible with the intestinal lesions found in cystic fibrosis (J. B. Arey, personal communication). Pancreatic changes were minimal and included dilatation of a few acini, some retention of secretions, and a small amount of interstitial fibrosis.

Patient No. 2, a male, was born in 1956. The father is Korean, the mother Japanese. The paternal and maternal grandparents are Korean and Japanese, respectively, and there is no known Caucasian ancestry. There is no consanguinity. There are two normal sibs. Birth weight was 6 pounds, 1 ounce. From birth there was chronic diarrhea and poor weight gain. The highest weight attained was 7 pounds. Stools were described as "watery." The baby was "always hungry." Two stools were "negative" for tryptic activity. Chest plate, spinal fluid, and urine were normal. On the day of admission to the hospital (at age 6 weeks), the baby's respiration was rapid and labored. He weighed 6 pounds, 1 ounce, and appeared chronically ill and malnourished; the abdomen was tympanitic and protuberant. Death occurred shortly after admission and before further studies could be carried out.

The significant findings at autopsy were as follows: The pancreas was replaced by a small collection of fibroadipose tissue. No heterotopic pancreatic tissue was found. The lungs were well aerated with a moderate degree of emphysema and there was no evidence of pneumonia. Gastric contents were present within the tracheobronchial tree. Microscopic sections revealed a small island of pancreatic tissue at the head of the pancreas with increase in interstitial fibrous connective tissue and dilatation of the ductal structures. The lesions were compatible with those found in cystic fibrosis (J. B. Arey, personal communication). The islet cells were intact. No pancreatic tissue could be identified in the fibrous tissue which had replaced the body and tail of

the pancreas. Sections from the lungs revealed a patchy emphysema without evidence of inflammatory reaction. Aspiration material was present in the bronchioles. Sections from the salivary glands were normal.

Segregation Analysis

Twenty-four patients with CF were identified as having been born or having had the diagnosis of CF first made in Hawaii during the years 1950–65. Twenty-one patients were pure Caucasian. One was the product of a Caucasian father and half-

TABLE 3

s, *r*, *a*, AND *t* TABLE FOR CF DATA

Family No.	Sibship Size (s)	Number Affected (r)	Number of Probands (a)	Number of Ascertainments	
				Proband 1 (t_1)	Proband 2 (t_2)
1	1	1	1	1	0
2	1	1	1	2	0
3	1	1	1	3	0
4	2	1	1	1	0
5	2	1	1	2	0
6	2	1	1	2	0
7	2	1	1	3	0
8	2	1	1	3	0
9	2	1	1	3	0
10	2	2	2	1	1
11	2	2	2	5	5
12	3	1	1	4	0
13	3	1	1	3	0
14	3	1	1	2	0
15	4	1	1	4	0
16	4	2	2	3	3
17	5	1	1	3	0
18	5	2	1	2	0
19	6	3	2	4	1
20	7	1	1	3	0
Total	59	26	24	54	10

Caucasian, half-Japanese mother, and two were non-Caucasians. Information on the latter two infants is given above.

Segregation analysis was performed on these 20 families. Table 3 gives the *sr* table of r affected among s children, the *ra* table of a probands among r affected sibs, and the t table of ascertainments per proband (Morton, 1962). These data were analyzed for incomplete ascertainment in terms of three parameters: p, the recurrence risk in sibs of probands, assumed to be 1/4 as for a fully penetrant recessive gene; x, the proportion of sporadic cases of different etiology, assumed to be zero; π, the ascertainment probability, or the chance that an affected child in the population at risk will be a proband.

Iteration of π gave the maximum likelihood estimate $\pi = .901 \pm .032$, at which value the scores (U) and their variances (K) are:

$$U = \begin{bmatrix} -2.35 \\ 2.12 \\ -0.03 \end{bmatrix}; \qquad K = \begin{bmatrix} 152 & -60 & -21 \\ & 28 & 8 \\ & & 981 \end{bmatrix}.$$

There is no significant deviation from the null hypothesis that $p = 1/4$ and $x = 0$ in this small body of data.

To test further the possibility of sporadic cases, we analyzed two published studies (Crow, 1965; Danks *et al.*, 1965). The *sr*, *ra*, and *t* tables are shown in Table 4. The estimated ascertainment probabilities are $\pi = .616 \pm .026$ for the material of Danks *et al.* (1965) and $\pi = .349 \pm .080$ for the data of Lobeck, reported by Crow (1965). These values are lower than were found in the present study, which testifies to the more complete ascertainment possible in island populations. Crow did not report the *t* table of numbers of ascertainments per proband, and therefore the standard error of the estimate of π from his material is large.

At the maximum likelihood estimates of π, the total scores and covariances for the three studies are:

$$U = \begin{bmatrix} 35 \\ -9 \\ 0 \end{bmatrix} \text{ and } K = \begin{bmatrix} 3589 & -1075 & -394 \\ & 457 & 127 \\ & & 2575 \end{bmatrix}.$$

The estimated segregation frequency is $p = 0.2599 \pm 0.0168$, which agrees well with the expected value of .25, and the estimate of x is -0.02 ± 0.047, which gives no evidence for sporadic cases in this predominantly Caucasian material.

Gene Frequency and Incidence

Maximum likelihood estimates of gene frequencies in Caucasians and non-Caucasians and incidence in hybrids may be obtained as follows: Let the gene frequency be Q in Caucasians and q in non-Caucasians. Let C_M and C_F be the proportions of Caucasian ancestry in mothers and fathers, respectively, of a particular mating type. Then

$$X = C_M C_F$$

is the expected proportion of Caucasian homozygosity in the children, and

$$Z = C_M(1 - C_F) + C_F(1 - C_M)$$

is the expected proportion of Caucasian $\times$ non-Caucasian hybridity.

In mating type i, let there be n_i probands among N_i births at risk. The expected proportion is

$$p_i = E(n_i/N_i) = \pi[Q^2X + QqZ + q^2(1 - X - Z)].$$

The maximum likelihood score with respect to p_i is

$$u_{p_i} = 1/(p_i)$$

for each proband and

$$u_{p_i} = -1/(1 - p_i)$$

TABLE 4

sr, *ra*, AND *t* TABLES FOR SERIES REPORTED BY DANKS *et al.* AND CROW

DANKS *et al.* (1965)			CROW (1965)		
sr Tables					
Number of Sibships (*N*)	Sibship Size (*s*)	Number Affected (*r*)	Number of Sibships (*N*)	Sibship Size (*s*)	Number Affected (*r*)
19	1	1	9	1	1
39	2	1	18	2	1
11	2	2	6	2	2
48	3	1	10	3	1
19	3	2	6	3	2
1	3	3	.....	.....	.....
22	4	1	6	4	1
16	4	2	4	4	2
4	4	3	3	4	3
1	4	4	.....	.....	.....
10	5	1	2	5	1
10	5	2	5	5	2
6	5	3	2	5	3
5	6	1	1	6	1
5	6	2	1	6	2
1	6	3	.....	.....	.....
2	6	4	.....	.....	.....
2	7	1	1	7	1
2	7	2	1	7	2
2	7	3	2	7	3
2	7	4	.....	.....	.....
1	8	1	.....	.....	.....
1	8	2	1	8	4
1	9	3	1	9	3
1	10	3	1	10	3
1	10	4	.....	.....	.....

ra Tables					
Number of Sibships (*N*)	Number Affected (*r*)	Number of Probands (*a*)	Number of Sibships (*N*)	Number Affected (*r*)	Number of Probands (*a*)
146	1	1	47	1	1
37	2	1	18	2	1
27	2	2	5	2	2
8	3	1	5	3	1
6	3	2	3	3	2
2	3	3	1	3	3
3	4	1	1	4	1
2	4	2	.....	.....	.....
1	4	3	.....	.....	.....

t Table		
Number of Times Ascertained	Number of Cases	Total Ascertainments
1	96	96
2	35	70
3	5	15
4	2	8
Total	138	189

t Table
(Data not given.)

for each of $N_i - n_i$ nonprobands. Treating π as a known constant with negligible error,

$$u_q = u_p(dp/dq) = u_p\pi[2q(1 - X) + (Q - 2q)Z] ;$$

$$u_Q = u_p(dp/dQ) = u_p\pi(2QX + qZ) .$$

Starting with trial values of $q = .00453$ and $Q = .01606$ from the unmixed groups, we arrived, after several iterations, to the maximum likelihood solutions

$$\begin{pmatrix} q \\ Q \end{pmatrix} = \begin{pmatrix} .00331 \\ .01616 \end{pmatrix} + UK^{-1} ,$$

where

$$U = \begin{pmatrix} \Sigma u_q \\ \Sigma u_Q \end{pmatrix} = 0 ,$$

and

$$K = \begin{pmatrix} \Sigma u_q^2 & \Sigma u_q u_Q \\ & \Sigma u_Q^2 \end{pmatrix} = \begin{pmatrix} 11853 & 6 \\ & 335 \end{pmatrix} \times 10^3 .$$

TABLE 5

INCIDENCE OF CYSTIC FIBROSIS FOR LIVE BIRTHS IN HAWAII, 1950–1965

MATING TYPE AS A PROPORTION OF CAUCASIAN ANCESTRY	X	Z	NUMBER LIVE BIRTHS AT RISK 1950–1965 (N)	NUMBER OF PROBANDS		ESTIMATED INCIDENCE PER 100,000 LIVE BIRTHS AT RISK $= [E(n)/\pi N] \times 10^{-5}$
				Observed (n)	Expected ($E[n]$)	
0×0	0	0	96,968	2	0.96	1.10
$0\times\frac{1}{4}$	0	1/4	9,376	0	0.18	2.13
$0\times\frac{1}{2}$	0	1/2	14,952	0	0.43	3.19
0×1	0	1	14,368	0	0.69	5.33
$\frac{1}{4}\times\frac{1}{4}$	1/16	3/8	1,334	0	0.05	4.16
$\frac{1}{4}\times\frac{1}{2}$	1/8	1/2	4,897	0	0.28	6.35
$\frac{1}{4}\times1$	1/4	3/4	2,441	0	0.23	10.46
$\frac{1}{2}\times\frac{1}{2}$	1/4	1/2	6,923	0	0.59	9.46
$\frac{1}{2}\times1$	1/2	1/2	9,616	1	1.36	15.70
1×1	1	0	81,479	21	19.16	26.11
Total	.....	.....	242,354	24	24.00	.....

NOTE.—$\pi = .90062$, $X = C_M C_F$, and $Z = C_M(1 - C_F) + C_F(1 - C_M)$ (see text).

The gene frequency difference between Caucasians and non-Caucasians $(Q - q)$ has the variance

$$\frac{\Sigma u_Q^2 + \Sigma u_q^2 + 2\Sigma u_q u_Q}{(\Sigma u_Q^2)(\Sigma u_q^2) - (\Sigma u_q u_Q)^2} = 3.07 \times 10^{-6}$$

and is therefore highly significant ($\chi^2_{[1]} = 54$, $P < .001$).

Table 5 gives the incidence of CF in unmixed and hybrid children. The incidence and gene frequencies for pure Caucasian and pure non-Caucasian races are shown in Table 6.

The high rate of CF in Caucasians is strikingly reduced in hybrids with a non-Caucasian race. These changes are shown graphically in Figure 1.

DISCUSSION

The estimated incidence of CF—about one per 3,800 live births in the Caucasian population of Hawaii—is consistent with estimates made in other Caucasian groups (Steinberg and Brown, 1960; Kramm *et al.*, 1962; Merritt *et al.*, 1962; Danks *et al.*, 1965). The estimate for non-Caucasian populations represents the first such estimate to be made in these groups. Since the non-Caucasian groups in Hawaii are predominantly Oriental, that is, Japanese, Chinese, and Korean, one per 90,000 live births represents a reasonable estimate of the incidence of CF in an Oriental population. That the disorder is rare in these groups is also shown by the autopsy data: CF was about eight times more frequent in the Caucasian group.

The low incidence of CF in the non-Caucasian group is in striking contrast to the high value in Caucasians. The differences in the two populations may be related to the presence of different alleles, selection differential, or genetic drift. At present, it is not clear whether clinical differences exist between affected children from the two

TABLE 6

	Gene Frequency	Incidence per 100,000 Live Births
Caucasians	0.01616 ± 0.00173	26.11 (about 1:3,800)
Non-Caucasians	0.00331 ± 0.00029	1.10 (about 1:90,000)

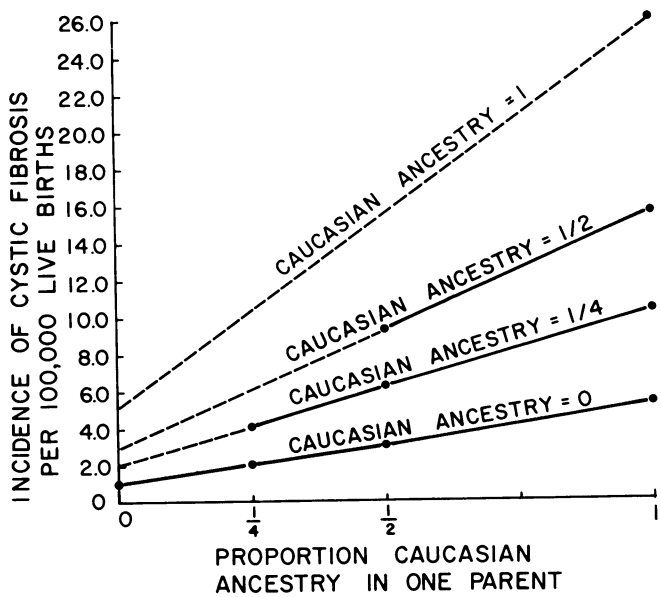

FIG. 1.—Incidence of CF per 100,000 live births in unmixed and hybrid matings. On the abscissa, the proportion of Caucasian ancestry may vary from zero to one for one of the parents. For the other parent, the proportion of Caucasian ancestry is fixed at one value.

populations. The available reports from Japan suggest a higher number of patients with meconium ileus and describe histological findings that are often suggestive but not conclusive (Yamamoto and Abe, 1957; Hamamoto *et al.*, 1961; Ikai *et al.*, 1965; Ikai, personal communication; and H. Schwachman, personal communication). Comparative studies of the histological findings in Caucasian and Japanese patients with meconium ileus as well as comparative clinical studies on infants identified through newborn screening programs will provide data for determining whether histological and clinical differences do exist.

Differentiation of populations is appropriately measured by the equivalent inbreeding coefficient (Cavalli-Sforza *et al.*, 1964; Morton *et al.*, 1967),

$$F = \frac{\sigma^2}{\bar{q}(1 - \bar{q})},$$

where $\bar{q}$ is a mean gene frequency and σ^2 is its variance among populations. For CF in Caucasians and non-Caucasians, we have

$$\sigma^2 = (Q - q)^2/4 = .000041 ;$$

$$\bar{q} = (Q + q)/2 = .009735 ;$$

and therefore $F = .0043$.

This value is greater than the equivalent inbreeding coefficient of .0009 estimated from morbidity in crosses between Caucasians and non-Caucasians, which has been attributed to the load of deleterious genes maintained by mutation (Morton *et al.*, 1967). The relatively high value for CF is suggestive of differential selection in Caucasians and non-Caucasians. Since affected homozygotes rarely survive to reproductive maturity, the postulated selection differential must favor heterozygotes in Caucasian populations. If more observations confirm the indication of unusually early mortality of non-Caucasian homozygotes, it will be tempting to speculate that heterozygote advantage was limited to a Caucasian allele which may arise so rarely by mutation as not to be available for concurrent selection in non-Caucasian populations. However, it is impossible at this point to rule out genetic drift as the cause of the high frequency in Caucasians. Nor is it possible to rule out the fact that the gene may have been introduced into the Oriental population by migration.

The complexity of this dilemma is revealed by computing the probability of obtaining the Caucasian gene frequency, $Q = .01616$, by genetic drift in an array of populations with equilibrium frequency $q = .00331$ (as for non-Caucasians) and inbreeding coefficient .0009 (as for other data on morbidity in Caucasian and non-Caucasian crosses in Hawaii). We assume that $y = \ln(Q/q)$ has a normal distribution with mean 0 and variance $\sigma^2/q^2 = (1 - q)F/q = .271$. For $Q = .01616$, the standardized normal deviate is $y/\sigma = 3.05$. Thus, the probability of $Q > .01616$ from this distribution is about .0012; so drift is unlikely to cause such a large difference in gene frequency as is observed between Caucasians and non-Caucasians. On the other hand, among 1,000 lethal recessive genes, we would expect at least one to diverge in frequency this much by drift alone, and CF may be just such a rare accident among the hundreds of recessive lethals known in man. Thus, it is not possible on present evi-

dence to decide with any assurance whether the high frequency of CF in Caucasians is due to drift or heterozygote advantage.

Recently, Knudson *et al.* (1967) compared the size of CF families with controls who were "for the most part friends and neighbors of the study group subjects." They took the precaution to exclude families with no children. Grandparents of CF children averaged 4.34 offspring compared to 3.43 for controls ($P < .01$). However, the control is not sufficiently comparable to relatives of a group under incomplete selection by which the probability of ascertaining a family varies with its size (Barrai *et al.*, 1965). Thus, evidence of heterozygote advantage remains unconvincing.

We have based our estimate of the mutation rate to alleles for CF on the non-Caucasian data. The quantity $z = q + h + a$ has been estimated as .01–.02 for lethal recessive genes and is perhaps closer to the smaller figure (Dewey *et al.*, 1965) where h is the dominance and a the mean inbreeding coefficient in the past. The corresponding mutation rate is $u = zq = 3.3 \times 10^{-5}$, a typical value entirely consistent with a mutational equilibrium in non-Caucasians.

A practical use of the data and methodology prescribed here concerns the estimation from Figure 1 of the number of patients with CF born in a hybrid population during a specified interval of time. This information is useful in estimating the results from newborn screening programs for CF in Hawaii. Simple techniques are now being developed for early recognition of CF in newborns (Warwick, 1966). The early institution of prophylactic measures may decrease both morbidity and mortality from the disease (Schwachman *et al.*, 1965). Further, the methodology for estimating gene frequency and incidence may have usefulness in estimating the results from screening programs for other autosomal recessive traits in hybrid populations, such as phenylketonuria, galactosemia, etc.

SUMMARY

The gene frequency estimates for cystic fibrosis in Hawaii are .00331 $\pm$ .00029 in non-Caucasians and .01616 $\pm$ .00173 in Caucasians, the corresponding incidences corrected for ascertainment being 1.10 and 26.11 per 100,000 live births for non-Caucasians and Caucasians, respectively. The equivalent inbreeding coefficient measuring this differentiation is $F = .0043$, which is substantially greater than for outcrossing effects on morbidity, mortality, and birth weight. These observations suggest that CF is maintained by recurrent mutation in non-Caucasians at the rate of about 3.3×10^{-5} mutations per locus per generation, but has been favored in Caucasian heterozygotes. The possibility is considered that the typical Caucasian allele may arise so rarely by mutation as not to have been available for selection in non-Caucasian populations, but the available evidence is inadequate to rule out the alternative hypotheses that the high frequency in Caucasians is due to genetic drift or to selective pressures that are not present in non-Caucasians.

ACKNOWLEDGMENTS

The authors are grateful for the assistance of the many pathologists, pediatricians, general physicians, record librarians, and administrators in the hospitals, the Hawaii State Department of Health, and the Cystic Fibrosis Foundation who made this study possible.

REFERENCES

BARRAI, I., MI, M. P., MORTON, N. E., and YASUDA, N. 1965. Estimation of prevalence under incomplete selection. *Amer. J. Hum. Genet.* **17**:221–236.

CAVALLI-SFORZA, L. L., BARRAI, I., and EDWARDS, A. W. F. 1964. Analysis of human evolution under random genetic drift. *Cold Spring Harbor Symp. Quant. Biol.* **29**:9–20.

CROW, J. F. 1965. Problems of ascertainment in the analysis of family data. Pp. 23–44 *in* J. V. NEEL, M. W. SHAW, and W. J. SCHULL (eds.), *Genetics and the epidemiology of chronic diseases.* U.S. Department of Health, Education, and Welfare, Washington.

DANKS, D. M., ALLAN, J., and ANDERSON, C. M. 1965. A genetic study of fibrocystic disease of the pancreas. *Ann. Hum. Genet.* (Lond.) **28**:323.

DEWEY, W. J., BARRAI, I., MORTON, N. E., and MI, M. P. 1965. Recessive genes in severe mental defect. *Amer. J. Hum. Genet.* **17**:237–256.

HALLETT, W. Y., KNUDSON, A. G., and MASSEY, F. J. 1965. Absence of detrimental effect of the carrier state for the cystic fibrosis gene. *Ann. Rev. Resp. Dis.* **92**:714–724.

HAMAMOTO, E., OHTAHARA, S., and IIZUKA, K. 1961. Cystic fibrosis of the pancreas in a Japanese child. *Acta Paediat. Jap.* **65**:502.

HARRIS, R. L., and RILEY, H. D., JR. 1968. Cystic fibrosis in the American Indian. *J. Pediat.* (in press).

IKAI, K., SUGIE, I., SUGINO, I., NITTA, H., IIDA, T., OGAWA, J., and SUECKI, T. 1965. Cystic fibrosis cases found by re-examination of histology of pancreas and post mortem protocol in Japanese children and sweat test on siblings. *Acta Paediat. Jap.* **7**:23.

KANDAR, H. R. 1961. Cystic fibrosis of the pancreas with respiratory allergy in children. *Antiseptic* **58**:863.

KNUDSON, A. G., JR., WAYNE, L., and HALLETT, W. Y. 1967. On the selective advantage of cystic fibrosis heterozygotes. *Amer. J. Hum. Genet.* **19**:388–392.

KRAMM, E. R., CRANE, M. M., SIRKEN, M. G., and BROWN, M. C. 1962. A cystic fibrosis pilot survey in three New England states. *Amer. J. Public Health* **52**:2041.

KULCZYCKI, L. L., GUIN, G. H., and MANN, N. 1964. Cystic fibrosis in Negro children. *Clin. Pediat.* **3**:692.

LEVIN, S. 1963. P. 148 *in* E. GOLDSCHMIDT (ed.), *Genetics of migrant and isolate populations.* Williams & Wilkins, Baltimore.

LOBECK, C. C. 1966. Cystic fibrosis of the pancreas. Pp. 1301–1316 *in* J. B. STANBURY, J. B. WYNGAARDEN, and D. S. FREDERICKSON (eds.), *The metabolic basis of inherited disease.* 2d ed. McGraw-Hill, New York.

MERRITT, A. D., HANNA, B. L., TODD, C. W., and MYDERS, T. L. 1962. Incidence and mode of inheritance of cystic fibrosis. *J. Lab. Clin. Med.* **60**:998.

MORTON, N. E. 1962. Segregation and linkage. Pp. 17–52 *in* W. J. BURDETTE (ed.), *Methodology in human genetics.* Holden-Day, San Francisco.

MORTON, N. E., CHUNG, C. S., and MI, M. P. 1967. *Genetics of interracial crosses in Hawaii. Monographs in human genetics.* Vol. 3. Karger, Basel. 160 pp.

SALEM, M., and IDRESH, H. 1962. Cystic fibrosis of the pancreas in the Middle East. *J. Med. Liban.* **15**:61.

SCHWACHMAN, H., KULCZYCKI, L., and KHAW, K. T. 1965. Studies in cystic fibrosis: a report on 65 patients over 17 years of age. *Pediatrics* **36**:689.

STEINBERG, A. G., and BROWN, D. C. 1960. On the incidence of cystic fibrosis of the pancreas. *Amer. J. Hum. Genet.* **12**:416–424.

THOMAIDES, T. S., and AREY, J. B. 1963. The intestinal lesions in cystic fibrosis of the pancreas. *J. Pediat.* **63**:444–453.

WARWICK, W. 1966. Cystic fibrosis sweat test for newborns. *J. Amer. Med. Ass.* **198**:177.

YAMAMOTO, T., and ABE, H. 1957. An autopsied case of meconium ileus (cystic fibrosis of the pancreas) in a Japanese child. *Shonika Rinsho* **10**:1003.