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THE LINKAGE ANALYSIS OF CYSTIC FIBROSIS AND RED AND WHITE CELL LOCI

By

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ABSTRACT

THE LINKAGE ANALYSIS OF CYSTIC FIBROSIS
AND RED AND WHITE CELL LOCI

By

Janel Susan Rehm Hector

Cystic fibrosis of the pancreas causes severe chronic pulmonary disease, dietary insufficiencies and delayed growth in infants, children and young adults. Inheritance is autosomal recessive. Heterozygote individuals cannot be distinguished currently from individuals homozygous for the nonaffected allele. The cystic fibrosis locus has not yet received chromosomal assignment, and is not known to be linked with any other locus of the human genome.

Affected individuals and their siblings, parents and grandparents were typed for antigens from the histocompatibility complex and nine of the red blood cell loci (ABO, Rhesus, MNSs, Kell, Kidd, P, Duffy, Lewis and Secretor). The resulting family data were analyzed for association of cystic fibrosis with the tested antigens (chi-square method), and linkage of the cystic fibrosis locus with the test loci (lod scores of Morton).

Linkage analysis excludes the cystic fibrosis locus from within .20 or less map units from the ABO locus, .13 or less units from the HLA loci, .09 or less units from the Rhesus loci, .08 or less units from the MNSs loci, .04 or less units from the Duffy and P loci and .01 or less units from the Kidd, Lewis and Secretor loci. Possible locations of the cystic fibrosis loci are at zero map units from the

Kell locus (lod scores of 2.0727) with a probability of 118 to 1 and .30 map units from the MNSs loci (lod score of 1.781) with a probability of 60 to 1.

Association analysis indicated a very high association between the HLA-B7 antigen of the histocompatibility complex and the occurrence of cystic fibrosis. Of 67 cystic fibrosis children, 13 had the HLA-B7 antigen and 54 did not have the antigen; 25 of a total of 51 non-affected siblings possessed the HLA-B7 antigen. (Chi-square value of 11.6339, probability between .0010 and .0005, highly significant.) The HLA-B27 antigen was not significantly associated with the occurrence of cystic fibrosis. Four of the 67 cystic fibrosis individuals possessed HLA-B27; 7 of 51 full siblings were HLA-B27 positive (chi-square value of 2.0604, probability between .2 and .1), no significance.

Dedicated to
my husband
David Alphonso Hector, M.D.
with love

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I. BACKGROUND

A. INTRODUCTION

The purpose of this study was to identify any antigen association or linkage relationship between cystic fibrosis and ten of the red and white blood cell loci: HLA (the human histocompatibility complex), ABO, Rhesus, MNSs, Kell, Duffy, Kidd, P and Lewis-Secretor).

Association of cystic fibrosis with the blood cell antigens was tested by chi-square analysis, with the full siblings of the propositus (cystic fibrosis) individuals as a control group.

Linkage was tested between cystic fibrosis and the ten test loci using the log-odd (lod) score method of Morton; grandparents were tested to aid in identification of recessive alleles in the parental genotypes.

A genetic linkage of a cystic fibrosis locus with a test locus could provide the basis for heterozygote identification and the consequent prenatal diagnosis of cystic fibrosis in affected families.

B. CYSTIC FIBROSIS OF THE PANCREAS

Cystic fibrosis was recognized as a separate disease entity in the 1920's based on characteristic findings at autopsy, including fibrosis of the pancreas. In the 1930's cystic fibrosis was defined clinically.[1] Until 1945, the average age of death of children diagnosed as having cystic fibrosis was one year.[1]

Cystic fibrosis is an inherited autosomal recessive disease characterized by chronic pulmonary disease, chronic maldigestion and malabsorption and an elevated sweat chloride level. Males and females are equally affected.[1] Ratios of affected to normal children in sibships correspond to expected Mendelian ratios of one to three when a correction is made for those sibships where no cystic fibrosis individuals occur.[2]

The child with cystic fibrosis has two recessive genes that produce the disease state. The phenotypically normal parents are carriers, or heterozygotes at the cystic fibrosis locus. Any child of these parents has a one-in-four chance of receiving a cystic fibrosis gene from both parents and having clinical disease. A phenotypically normal child from the same mating may be a carrier for the allele or may have two nonaffected alleles. Each phenotypically normal child has a two to one probability of being a carrier of the cystic fibrosis allele, and one chance in 120 of producing a cystic fibrosis child from a random mating with another phenotypically normal individual. Attempts at heterozygote assays are currently unable to distinguish between the normal and carrier siblings. (Tables 1 and 2)

As a result of the life-limiting nature of the disease process, few cystic fibrosis individuals live long enough to reproduce. Those children born to cystic fibrosis individuals may be homozygous for cystic fibrosis or may be heterozygous depending on the genotype of the other parent. Children of an affected individual and a carrier will have a fifty percent chance of being affected; all phenotypically

TABLE 1. INHERITANCE OF CYSTIC FIBROSIS.

Mating Type	Genotype of Offspring
1. CfCf x CfCf	100% CfCf
2. CfCf x Cfcf	50% CfCf + 50% Cfcf
3. CfCf x cfcf	100% Cfcf
4. Cfcf x Cfcf	25% CfCf + 50% Cfcf + 25% cfcf
5. Cfcf x cfcf	50% Cfcf + 50% cfcf
6. cfcf x cfcf	100% cfcf

CfCf represents a nonaffected individual at the cystic fibrosis locus, Cfcf is a heterozygote or carrier, and cfcf is an individual affected with cystic fibrosis.

TABLE 2. RISK OF A NORMAL SIBLING (OF A CYSTIC FIBROSIS INDIVIDUAL) TO PRODUCE A CHILD AFFECTED WITH CYSTIC FIBROSIS.

Mating Type	Offspring	Probability of Mating
1. Sibling of CfCf, mate is CfCf	100% CfCf	(1/3) (19/20)
2. Sibling is CfCf, mate is Cfcf	50% CfCf, 50% Cfcf	(1/3) (1/20)
3. Sibling is Cfcf, mate is CfCf	50% CfCf, 50%Cfcf	(2/3) (19/20)
4. Sibling is Cfcf, mate is Cfcf	25% CfCf, 50% Cfcf 25% cfcf	(2/3) (1/20)

An affected child can occur only in mating type 4. The probability for a sibling of a cystic fibrosis individual to have an affected child is: (2/3) x (1/20) x (1/4) = 1/120

normal children will be carriers. A mating with an individual homozygous for the nonaffected allele will produce only heterozygous children. Based on racial gene frequencies, the probability of a cystic fibrosis offspring for an unknown mating has been approximated at two and one-half percent.[3] The phenotype of each successive child will give evidence of the genotype of the normal mate.

A mating between two affected individuals has never been reported but would be expected to result in all affected children. (This mating is unlikely not only because of the limited life span of affected individuals but also because affected males are usually sterile.)

Cystic fibrosis occurs primarily in Caucasian populations. (Table 3) The incidence of cystic fibrosis in Caucasians is estimated to be from one in 1000 to one in 3500 live births.[1] Based on the frequency of cystic fibrosis births, and using the Hardy-Weinberg law, $p + q = 1$, it can be estimated that approximately one person in twenty is a carrier.

In non-Caucasian populations, cystic fibrosis occurs rarely; the incidence of cystic fibrosis in Orientals residing in Hawaii is only one in 90,000 live births.[4] The incidence of cystic fibrosis in Native African Blacks is even lower; a search of world literature revealed only eighteen reported cases of cystic fibrosis in these non-American Blacks.[5] (These comparisons assume that diagnostic criteria for cystic fibrosis is the same in medical facilities

TABLE 3. POPULATION FREQUENCIES OF CYSTIC FIBROSIS.

Parental Ethnic Group	Affected newborns/live births
1. Caucasian	1/1000 - 1/3500
2. Mixed Caucasian - Oriental	1/14,000
3. Oriental	1/90,000
4. Non-American Blacks	rare*
5. Mixed Caucasian - Black	1/17,000

*Only eighteen cases reported in the world literature.

worldwide as it is in the clinics of the United States, and that homozygous cystic fibrosis alleles superimposed on different genetic backgrounds and in different geographic areas will still be expressed and clinically identifiable.)

Populations with mixed genetic backgrounds express intermediate frequencies of cystic fibrosis. For example, Hawaiian matings of mixed ancestry (i.e. both Oriental and Caucasian ancestors) have an average frequency of cystic fibrosis of one in 14,000 live births. [6]

It has been estimated, based on studies of the Rh blood groups, that approximately thirty percent of the alleles now present in the American Black population are of Caucasian origin.[2] The incidence of cystic fibrosis in a Black population in Washington, D.C. is about one in 17,000 live births;[5] this is intermediate between the incidence of cystic fibrosis in Caucasian populations and non-American Black populations.

For the Caucasian population, this information results in a genetic paradox. The cystic fibrosis allele is an almost complete genetic lethal - very few affected children survive to reproductive age, and of the survivors, most males are sterile. According to the Hardy-Weinberg law, $p^2 + 2pq + q^2 = 1$; if the q^2 or homozygous recessive individuals of each generation do not survive to reproduce, then the succeeding generations will result from matings within the remaining $(p^2 + 2pq)$ population representing homozygous normal and heterozygous individuals. With no other factors operating than normal random mating, the incidence of cystic fibrosis would be expected to be low,

and the cystic fibrosis allele would be maintained in the population by mutation. This may be the situation represented in the Oriental and non-American Black populations. However in the Caucasian population, the incidence of cystic fibrosis is too high to be explained simply by normal mutation rates.

Average mutation rates have been estimated from spontaneous new mutations of a number of dominant disorders, and may be approximated at from one to five per 100,000 genes.[7] For a recessive allele such as cystic fibrosis, the mutation rate, u, is equal to $(1 - f)(x)$,[7] where f is the relative fitness of cystic fibrosis individuals, and x is the frequency of cystic fibrosis. The fitness of cystic fibrosis individuals in terms of reproduction may be estimated as zero, and the frequency of cystic fibrosis in Caucasian populations may be taken as one in 1600. Therefore, the mutation rate of the normal allele at the cystic fibrosis locus to become a cystic fibrosis allele may be estimated at one in 1600, which is from 12.5 to 62.5 times greater than the average mutation rate of one to 5 in 100,000.

Possible explainations of the high frequency of cystic fibrosis in Caucasians include an unusually high mutation rate that is not present in other populations.[7] Alternatively, there may be a heterozygote advantage at the cystic fibrosis locus in Caucasians, not present in other populations.

It has been suggested that a selective advantage of heterozygotes may be manifested by increased numbers of offspring.[8] Greater numbers of offspring resulting from some undetermined reproductive advantage,

would cause greater numbers of cystic fibrosis children, and carrier siblings, thus increasing the frequency of the cystic fibrosis allele. Another suggested heterozygote advantage may be the reduction of blood pressure, due to sweat electrolyte loss, resulting in a decreased probability of hypertension in carriers[9] and therefore a longer reproductive period. Increased sibship size may also be attributed to compensation, by the parents, for the loss of affected offspring.

Clinically, affected individuals manifest a number of symptoms; whether these result from the presence of two recessive alleles for cystic fibrosis or from the absence of a dominant nonaffected allele at the cystic fibrosis locus is not yet known. A basic triad of symptoms - decreased pancreatic excretory function, pulmonary obstructive disease and increased sweat electrolytes - along with pedigree information, is considered diagnostic for cystic fibrosis.

The prenatal period is generally uneventful, and general physical examination of the neonate does not reveal any specific signs of cystic fibrosis. The first clinical indication of cystic fibrosis may be failure of the newborn to pass meconium. Usually the meconium is viscid and is unable to proceed past the terminal portion of the ileum (hence the term "meconium ileus"). Large amounts of protein in the meconium of affected individuals [10, 11, 12] may be related to obstruction as well as the metabolic defect of cystic fibrosis. Not only do 5 to 10 percent of cystic fibrosis patients have a history of neonatal meconium ileus[3], obstruction may also occur in

the older child (meconium ileus equivelant)[13]. The trapped meconium sometimes has to be removed surgically, however complete bowel obstruction seldom recurs.

Pulmonary disease is present in almost all affected individuals,[3] and may appear weeks or years after birth. The pulmonary disease results from continuous and viscid mucous production. Mild symptoms include a nonproductive cough; subsequent respiratory infections may result in the first signs of pulmonary obstruction. Repeated respiratory infections increase the obstruction and the compensatory emphysema results in the typical barrel shaped chest. Cor pulmonale and cardiac failure are often the end results of the chronic lung disease; other pulmonary complications include lobar atelectasis, lung abcesses, hemoptysis and pneumothorax.[3] Antibiotics are used to minimize respiratory infections and their consequences, and daily pulmonary drainage helps eliminate much of the mucous produced, slowing the progressive airway obstructive process.

Pancreatic insufficiency is present in eighty percent of cystic fibrosis patients.[3] Although endocrine function is generally intact, pancreatic enzyme production is severely limited, again due to mucoid obstruction of the pancreatic ducts. This results in maldigestion and malabsorption of food. Stools are large, bowel movements frequent, and the abdomen protruberant. These children may eat great amounts of food and yet be malnourished, exhibiting "failure-to-thrive" or stunted growth. Pancreatic enzymes can be given orally to increase intestional absorption although the pancreatic obstruction continues. Dietary supplements are also available

containing necessary calories and protein that do not require pancreatic enzymes for digestion.[14, 15, 16] Eventually, the fibrosis of the pancreas due to the mucous obstruction of the pancreas may hinder endocrine function and result in secondary diabetes mellitus.

Sweat electrolytes are often used as a diagnostic test for cystic fibrosis. The skin is salty to the taste due to increased sodium, chlorine and potassium. Excretory regulation of electrolytes is normal, however electrolyte loss from profuse sweating may result in cardiovascular collapse in hot weather (heat prostration).[3] A possible beneficial result of electrolyte loss is lowered blood pressure in affected individuals.[9]

Other less frequent clinical problems of cystic fibrosis children include rectal prolapse, abdominal adhesions, intussusception, hypoalbuminemia, lactase deficiency, neonatal obstructive jaundice, ocular lesions and infertility.[3] Cystic fibrosis may also coexist with other disease entities such as Kartagener syndrome[17], Silver-Russell dwarfism[18], leukemia[19], respiratory allergy[20], and chromosomal deletion syndromes[21].

Along with physical symptoms, patients with cystic fibrosis may have difficulties in dealing with personal, family and social problems due to the severity of the physical disease and the poor prognosis.[22] Intelligence of affected individuals is normal[22], and a high compliance rate during antibiotic treatment suggests that patients understand the seriousness of their disease process.[23] Pulmonary drainage is an

important part of treatment requiring assistance; this makes separation of the young adult patient from the parents and family difficult.[24]

The prognosis for patients with cystic fibrosis has always been poor. It is now estimated that twenty percent of all cystic fibrosis patients are fifteen years or older[1]; this increase in life expectancy may be attributed to a number of medical advances, and especially to the use of antibiotics in the treatment of respiratory infections, surgical intervention of meconium ileus and improved diet.

For the infant, meconium ileus may result in very early death. For survivors, respiratory obstruction and complications are the most significant threat to life, and the severity of the pulmonary disease generally determines the prognosis for the child. Early diagnosis and initiation of respiratory therapy offer the best hope at this time, and are aided by factors of general health such as diet and exercise.

Pancreatic insufficiency is now seldom a direct cause of death, because of enzyme supplements and artificial diets, however poor nutrition may predispose to other complications and decrease the patient's resistance to infections or other forms of stress.[26]

Occasional deaths occur due to other complications such as salt loss in hot weather, portal hypertension or diabetes.[26]

Increasing numbers of individuals with cystic fibrosis are now surviving into adulthood. This relatively long lifespan of these

individuals may be due to one or more of several factors - genetic make-up, environment, compliance and nature of treatment, nutrition and general health and emotional status. Males have a slightly longer life expectancy than females for unknown reasons. If cystic fibrosis is composed of more than one disease entity, as has been suggested[25], one form may be milder than another.

Reproduction of cystic fibrosis individuals is still rare due to the limited lifespan of affected individuals.[3] Although both males and females acheive sexual maturation, the time of maturation is frequently delayed in the more severely affected individuals. Most males (97 to 98%[26]) are sterile due to aspermia resulting from abnormal development of the epididymis, vas deferens and seminal vesicles[3]; however, several cases of fertile males have been reported.[3] Females are fertile and a number of successful pregnancies have been recorded, all resulting in normal offspring except one.[3, 25] However, pregnancy and delivery in cystic fibrosis females may be complicated by their pulmonary disease, and difficulties with the pregnancy are directly correlated with the degree of pulmonary involvement.[3]

C. ANALYSIS OF LINKAGE

The preferred method currently available for the analysis of human linkage data is the log-odd ratio or lod score developed by Morton.[27] These scores were developed based on two concepts: first, that the analysis of human linkage data is a sequential process, i.e. that

many families may be tested and each family yields a little more information, and second, that the best method for analyzing such a sequential sample is a sequential probability ratio test.[28]

A lod score (z score) is calculated for each family using a formula chosen based on the mating type of the parents. (Tables 4 and 5) A correction score (s score) may also be added to the lod score if the genotype of one or both of the parents was known because of the phenotype of any one of the children. Z and c scores are calculated for each family added to the study. The equations for determining lod scores have two variables: first, the numbers of each possible kind of offspring in that family (a through h), and second, the chromosome map distance (theta) the researcher wishes to test. The scores of many families are additive at a given map distance. Further, the scores may be added at arbitrary intervals during the course of the linkage study. If, at a given map distance, the additive scores of a series of families is greater than +3, linkage is considered present between the test locus and the main locus. If the additive scores are less than -2, linkage is considered absent from the two loci for that map distance. Linkage is not excluded, however, from the two loci for greater or smaller map distances. The third possibility, that the additive score for a given map distance falls between -2 and +3, conceeds only that more data is needed, before a conclusion may be reached.

Each cumulative ("total") score contains three component parts: 1) male scores, 2) female scores and 3) both or combined scores.

TABLE 4. PARENTAL MATING TYPES AND z SCORES.

	Mating Type[1]	z Score[2]	Progeny Phenotype[3] a	b	c	d	e	f	g	h
1.	CfcfTt x Cfcftt	z2	CfT	cfT	Cft	cft	-	-	-	-
2.	CfcfT1T2 x CfcfT1T1	z2	CfT1	cfT1	CfT1T2	cfT1T2	-	-	-	-
3.	CfcfTt x CfcfTt	z3	CfT	Cft	cfT	cft	-	-	-	-
4.	CfcfT1T2 x CfcfT1T2	z4	CfT1	cfT1	CfT2	cfT2	CfT1T2	cfT1T2	-	-
5.	CfcfT1T2 x CfcfT1t	z6	CfT1	cfT1	CfT2	cfT2	CfT1T2	cfT1T2	-	-
6.	CfcfT1T2 x CfcfT3T4	z7	CfT1T3	cfT1T3	CfT2T3	cfT2T3	CfT1T4	cfT1T4	CfT2T4	cfT2T4

1. All parents of cystic fibrosis children are assumed to be heterozygotes and therefore Cfcf genotype. The genotype of the test locus is indicated by the T designation, where T1, T2, T3 and T4 are codominant, and t is recessive to T, T1, T2, T3 and T4.

2. See Table 5.

3. The phenotype of the progeny is given at both the cystic fibrosis and the test loci. The classifications a through h are used as variables in the z score formulas (Table 5).

TABLE 5. Z SCORE FORMULAS.[27, 30]

$$z2 = \log\ (2^{s-1}/3^{a+c})[(2-\theta)^{a}\theta^{b}(1+\theta)^{c}(1-\theta)^{d} + (1+\theta)^{a}(1-\theta)^{b}(2-\theta)^{c}\theta^{d}]$$

$$z3 = \log\ (4^{s-1}/9^{a}3^{b+c})[(3-2\theta+\theta^{2})^{a}\theta^{b+c}(2-\theta)^{b+c}(1-\theta)^{2d} + 2\{2+\theta-\theta^{2})^{a}(1-\theta+\theta^{2})^{b+c}\theta^{d}(1-\theta)^{d}$$
$$+ (2+\theta^{2})^{a}(1-\theta^{2})^{b+c}\theta^{2d}]$$

$$z4 = \log\ (4^{s-1}/3^{a+c+e}2^{e+f})[2^{e+f}\theta^{2b+c+f}(1-\theta)^{a+2d+f}(1+\theta)^{a}(2-\theta)^{c}(1-\theta+\theta^{2})^{e}$$
$$+ 2\theta^{b+d}(1-\theta)^{b+d}(1-\theta+\theta^{2})^{a+c}(1+2\theta-2\theta^{2})^{e}(1-2\theta+2\theta^{2})^{b}$$
$$+ 2^{e+f}\theta^{a+2d+f}(1-\theta)^{2b+c+f}(1+\theta)^{c}(2-\theta)^{a}(1-\theta+\theta^{2})^{e}]$$

$$z6 = \log\ (4^{s-1}/2^{a+b}3^{a+c+e})[(2-\theta)^{a+c}\theta^{b+c+f}(1-\theta)^{2d+f}(1-\theta+\theta^{2})^{e} + (2-\theta)^{a+c}\theta^{b+d+e}(1-\theta+\theta^{2})^{c}(1-\theta)^{d+2f}$$
$$+ (1+\theta)^{a}(1-\theta)^{b+d}(1-\theta+\theta^{2})^{c}\theta^{d+2f}(1-\theta^{2})^{e} + (1+\theta)^{a}(1-\theta)^{b+f}(1-\theta^{2})^{c}\theta^{2d+f}(1-\theta+\theta^{2})^{e}]$$

$$z7 = \log\ (4^{s-1}/3^{a+c+e+g})[(1-\theta^{2})^{a}\theta^{2b+d+f+g}(1-\theta+\theta^{2})^{c+e}(1-\theta)^{d+f+2h}(2-\theta)^{g}$$
$$+ (1-\theta+\theta^{2})^{a+g}\theta^{b+c+2f+h}(1-\theta)^{b+2d+h}(2-\theta)^{c}(1-\theta^{2})^{e}$$
$$+ (1-\theta+\theta^{2})^{a+g}\theta^{b+2d+e+h}(1-\theta)^{b+2f+h}(1-\theta^{2})^{c}(2-\theta)^{e}$$
$$+ \theta^{a+d+f+2h}(2-\theta)^{a}(1-\theta)^{2b+d+f}(1-\theta+\theta^{2})^{c+e}(1-\theta^{2})^{g}]$$

θ = map distance in Morgans, s = total number of children in cystic fibrosis sibship, and a, b, c ... h = number of children of each phenotype (see Table 4).

Families in which segregation is visible in the mother but not the father are viewed independently of families in which segregation of alleles are visible in the father only. This segregation results from linkage data (such as of the Lutheran and Secretor loci in man) indicating that recombination events occur more frequently in females than in males, and therefore that linkage might be detected more easily in males.[31, 32] In the families where segregation was visible in both parents, scores were also broken down into scores for female and for male segregation.

The null hypothesis being tested is that the recombination fraction of the two loci in the population is one-half. This situation would be present if: 1) the two loci were on different chromosomes (not linked), or 2) if the two loci were on the same chromosome but far enough apart so that the frequency of recombination events make the loci appear to assort independently (syntenic). Any variation from the appearance of no linkage, such as incomplete penetrance, biased family selection or ascertainment, non-random segregation of loci on nonhomologous chromosomes, or the presence of linkage between the two tested loci, may result in rejection of the null hypothesis.[27] Assumptions necessary for the application of the lod score test for linkage are: 1) the parental genotypes are known (except for phase), 2) the segregation ratios are not altered by incomplete penetrance or differential visibility, and 3) families are selected and ascertained randomly.[27] The truncate method of selection used to ascertain cystic fibrosis families is corrected for in the calculations.

When linkage is shown to be present at a tested map distance, the final step is to find the map distance of the two loci. The lod score sums may be graphically portrayed to visually find the most likely peak value; then the families may be scored at smaller intervals (such as one centimorgan map distance) to determine the peak value. Linkage distance varies between male, female, combined and total scores, and would be expected to be closer in male than in female scores.

While it is desirous to extend the human linkage map as quickly as possible, only those linkages which are certain should be added to the human gene map. On the other hand, there is less danger in incorrectly excluding a positive linkage due to nonrandom sampling or other statistical error. The limiting values of +3 and -2 correspond to type I and type II errors, respectively. The probability of a type I error (accepting a linkage that is in fact not present) is .001. The probability of a type II error (rejecting a linkage that is in fact present) is .01.[27] (Table 6)

D. DETECTION OF CARRIERS OF THE CYSTIC FIBROSIS ALLELE

Finding a consistent cystic fibrosis heterozygote assay is difficult because the biochemical defect for cystic fibrosis is unknown. The best known assay to date is the ciliary inhibition test, based on the observation that a protein in the serum of affected individuals and heterozygotes inhibits the beating action of cilia of rabbit trachea or oyster gills. This assay has been extremely difficult to standardize due to the problems associated with using a live organism

TABLE 6. TYPE A AND B ERRORS.[27]

α = .001, the probability of establishing a linkage which does, in fact, not exist.

β = .01, the probability of excluding a linkage which does, in fact, exist.

Log A = Log $[(1-\beta)/\alpha]$

Log A = +3, the minimum lod score accepted to confirm the presence of linkage.

Log B = Log $[\beta/(1-\alpha)]$

Log B = -2, the minimum lod score accepted to confirm the absence of linkage.

assay. The results have not been consistent when performed by different technicians or in different laboratories, thus limiting its potential diagnostic power for heterozygotes. Nor is ciliary dyskinesis specific to cystic fibrosis - other diseases of pulmonary or immunologic origin also inhibit ciliary movement,[33] thus complicating the diagnosis of a possibly heterozygous individual.

Other heterozygote assays include the staining patterns of leukocytes and fibroblasts. One study noted two types of families, those in which both affected individuals and parents leukocytes stained, and those in which cells of neither parents nor affected children stained.[34] No distinction was made between heterozygotes and nonaffected. Another study showed that cellular staining of fibroblasts also occurs in an array of different diseases, thus further reducing its effectiveness as a tool to diagnose cystic fibrosis heterozygotes.[35] Studies of leukocyte degranulation[36], isoelectic focusing of plasma[37] and of serum[38], beta-glucuronidase activity of sweat[39], and spermidine to spermine ratios in blood[40] also showed no significant difference between affected individuals and heterozygotes.

A number of other assays have been able to show a range of results, with heterozygotes reacting intermediately between affected and normal groups, but have been unable to specify accurately the genotype for any given individual. Some of these assays are based on determination of serum alpha-fetoprotein levels[41], serum complement three levels[42], plasma kallikrein activity[43], serum isoelectric focusing in thin-layer polyacrylamide gels[44], white cell glutathione reductase activity[45], and trypsin binding by alpha-2-macroglobulin[46].

E. CHROMOSOMAL ASSIGNMENT OF CYSTIC FIBROSIS

At the present time, the chromosomal location of the cystic fibrosis locus is unknown; nor is the cystic fibrosis locus known to be linked to any other locus of the human genome.

Chromosomal assignment of currently known loci depends on three basic techniques: 1) cell hybridization, 2) chromosomal rearrangements and 3) linkage studies. None of these techniques have yet been successful with the cystic fibrosis locus. First, cell hybridization studies provide assignments when specific chemical characteristics of a locus are known; however, no such metabolic alteration has yet been recognized in cystic fibrosis. Second, individuals expressing cystic fibrosis and a chromosomal anomaly simultaneously may give clues to the chromosomal location of the cystic fibrosis locus. One such child expressed both cystic fibrosis and Cri-du-chat syndrome.[21,47] Both parents and two siblings had normal phenotypes and karyotypes. A third sibling died at two days of age from respiratory insufficiency, but did not have meconium ileus and was not diagnosed as having cystic fibrosis. The determination must be whether the deletion child was "hemizygous" (because of the deletion) or homozygous for cystic fibrosis, and without a consistent heterozygote assay to test the parents, or a positive family history of cystic fibrosis such a distinction is not possible. Lastly, the cystic fibrosis locus may be shown to be linked to another locus which has already received chromosomal assignment.

Several studies have tested linkage of the cystic fibrosis locus to other loci. (Table 7) Fifty-eight families were scored for

TABLE 7. LOCI TESTED FOR LINKAGE WITH CYSTIC FIBROSIS,[29, 30, 48]

Test Locus	# Families	Visible Segregation	θ, Map Distance	Combined Lod Score
ABO	75	Male	0.10	-2.605
			0.30	-0.177
Duffy	56	Male	0.10	-0.290
			0.30	+0.222
HLA	33	Male	0.10	+0.584
			0.30	+0.439
Kidd	33	Male	0.10	+0.064
			0.30	-0.020
Kell	17	Male	0.10	-1.154
			0.30	-0.225
MNSs[1]	32	Male	0.10	+0.096
			0.30	+0.315
MNSs[2]	7	Male	0.10	+0.058
			0.30	+0.019
MNSs[3]	58	Male	0.05	-1.4264
			0.10	-0.5130
			0.30	+0.1163
		Female	0.05	+0.5133
			0.10	+0.7713
			0.30	+0.4303
		Both	0.05	-0.9131
			0.10	+0.2583
			0.30	+0.5466
		Total	0.05	-2.6306
			0.10	-0.2741
			0.30	+0.5279

1. These families were typed for the M, N and S antigens of the MNSs blood group.

2. These families were typed for the M, N, S and s antigens of the MNSs blood group.

3. All of these families were typed for the M and N antigens, and some were also tested for the S antigens.

linkage between the cystic fibrosis and the MNSs blood group loci; linkage was excluded for a map distance of less than five centimorgans, but the data was inconclusive for greater distance.[29, 30]

A collection of computer analyzed data has also been made available.[48] (Table 7) Linkage between the cystic fibrosis and ABO blood group loci was excluded at ten map units but inconclusive at thirty map units. Linkage was inconclusive at both ten and thirty map units for the cystic fibrosis locus and the Duffy locus (fifty-six families), the HLA loci (thirty-three families), the Kidd locus (thirty-three families), the Kell locus (seventeen families) and the MNSs locus (thirty-nine families). All of the above families were scored for segregation of the father only. (Data for map distances other than 10 and 30 map units were not published).

F. THE TEST LOCI

1. The Human Histocompatibility Region

The HLA loci are located on the short arm of the sixth human chromosome.[49, 50] The three loci to be tested in this study, A, B and C, are located along a 1.0 centimorgan chromosomal segment;[49, 51, 52, 53] the order of these loci from the centromere is HLA-B - HLA-C - HLA-A. All three loci are polymorphic and the A, B and C loci may be typed for 18, 32 and 8 alleles, respectively.[54] On a single chromosome, the alleles occurring at the A, B and C loci are called a haplotype; each individual has a pair of number six chromosomes, and therefore two HLA haplotypes. Typing of a single individual will result in six HLA antigens, two for each of the three loci. These six alleles may

be defined as two haplotypes by also typing other family members and correlating information on like and unlike antigens in each person. For example, a child has HLA antigens A1, A2, B5, B7, C1 and C4. The mother has antigens A1, A30, B5, B12, C3 and C4; the father has antigens A2, A33, B7, B35, C1 and C3. The child and mother have HLA antigens A1, B5 and C4 in common, and share the B5-C4-A1 haplotype. The father and child share the HLA haplotype B7-C1-A2. The mother has HLA antigens A30, B12 and C3 not shared by the child; these three antigens form her second HLA haplotype B12-C3-A30. The second chromosome of the father has the HLA haplotype B35-C3-A33.

Confusion in HLA typing may arise from several sources. Recombination events between the HLA-B and HLA-A loci are infrequent, but when present may cause some difficulty in assigning haplotype arrangements in family members. Occasionally, an individual has a homozygous phenotype at one or more of the loci; i.e., that person may have two identical alleles at the HLA-A locus. This possibility may be confirmed by typing of the parents and finding that both parents also have that particular allele. If one parent lacks that allele, the child may have a "blank" - an allele not yet identifiable by currently available antisera. Unidentified alleles represent approximately 1, 4 and 20 percent of the total alleles present in the Caucasian population for the HLA-A, B and C loci, respectively.[55] Other racial groups share many of the same alleles with Caucasians but have larger percentages of blanks at each locus.[49] An alternative to the presence of an unidentified allele or blank, is the possibility of a chromosomal deletion of that locus.

Although the HLA alleles are generally co-dominant in their behavior, a few antigens show enough cross-reactivity with antibodies of other alleles to be considered subgroups of a common antigen. Those similar alleles can be distinguished by using non-crossreacting antibodies.

Cystic fibrosis has not at this time been consistently associated with any one HLA antigen. Statistically significant increases in the frequency of the HLA-B18 antigen (in a population of 12 cystic fibrosis patients and 32 heterozygotes) in one study suggest a possible association.[56] However, this conclusion has not been supported by other larger studies. HLA frequencies of 28 patients and 240 unrelated blood donors showed no significant difference.[57, 58]

2. The Red Blood Cell Loci

The Rhesus system is composed of three loci, D, C and E (in that order). These loci are located on the distal end of the short arm of the first human chromosome.[50] Two common antigens, "D" and "d", determine whether a person is Rh positive or negative; the d allele is recessive and is manifested as the absence of "D" antigen. A third allele "D^u" occurs infrequently and represents a weak production of the D antigen. "D^u" is recessive to "D" but dominant to "d". The "C" and "c" alleles of the C locus and the "E" and "e" alleles of the E locus are codominant. As in the HLA system, the arrangement of D, C and E alleles on each first chromosome represents a Rhesus "haplotype" which can be confirmed by typing of other

family members. The "haplotypes" are often abbreviated by symbols such as R_1 (alleles D, C and e) or R_2 (alleles D, c and E). Recombination events occur very rarely between the three alleles, and the three Rhesus genes are considered inherited as a single complex.[59]

The ABO locus is located on the distal end of the long arm of the ninth human chromosome.[50, 60] Four common alleles are known: A_1, A_2, B and O. The O allele is recessive to the other three and the B allele is codominant with A_1 and A_2. The A_1 allele is dominant to the A_2 allele, and A_2 directs a decreased production of the A_1 antigen.

The Kell locus has two common codominant alleles, K and k. The chromosomal location of the Kell locus is unknown.

The Kidd locus has two common codominant alleles Jk^a and Jk^b, and is located on the long arm of chromosome 7.[61] A third allele Jk is recessive and rare in the Caucasian population.

The Duffy locus also has two common codominant alleles, Fy^a and Fy^b, and a recessive allele Fy which is not uncommon in the Caucasian population. The Duffy locus is located in the center of the long arm of the first chromosome.[62] Although Duffy and Rhesus are both located on the first human chromosome and therefore syntenic, they appear to be unlinked by family studies as a result of the map distance of more than fifty centimorgans (the limiting distance for family linkage studies).

The Lewis and Secretor loci are not linked but do interact to produce a single phenotypic response. The Lewis and Secretor loci both have two alleles which are of simple dominant-recessive inheritance. The alleles Le, le, Se and se may be present in an individual in one of nine combinations. The presence of dominant alleles of both loci (LeLeSeSe, LeLeSese, LeleSeSe or LeleSese) is represented by a phenotype of Le(a-b+). The presence of the dominant allele of the Lewis locus only (LeLesese or Lelesese) is represented by the Le(a+b-) phenotype. The absence of dominance at the Lewis locus (leleSeSe, leleSese or lelesese) is represented by the le(a-b-) phenotype. Neither the Lewis nor the Secretor loci have received chromosomal assignment.

The MN and Ss loci are closely linked and all four alleles (M, N, S and s) behave as codominants. As for the HLA and Rhesus systems, a chromosomal type may be defined after typing informative family members. The MNSs loci are located on the long arm of chromosome 4.[60]

The P locus is located on human chromosome six and is syntenic with (but not linked to) the HLA locus.[60] Two common alleles are present, P_1 and P_2; P_2 is recessive to P_1.

G. THE HUMAN GENOME

The total autosomal map distance has been estimated at 27.5 Morgans in the human male and 38.5 Morgans in the female.[63] In this study, the linkage of the cystic fibrosis locus will be tested with ten unlinked test linkage groups. The test loci to be typed in this

study are the HLA system (A, B and C loci), the ABO, Kell, Kidd, Rhesus, Duffy, Lewis (and Secretor), MNSs and P blood group loci, and the alpha-1-antitrypsin locus. (Table 8) Linkage will be tested at the practical limit of 0.40 Morgans map distance from each test locus. Thus a total of 8 Morgans map distance of the human autosome will be tested for presence of the cystic fibrosis locus. This represents a total of 29.1% and 20.8% of the total autosomal map distance in the male and female, respectively. The total map distance these test loci have excluded from linkage with cystic fibrosis from prior studies is only 0.30 Morgans, or 1.1% and 0.8%, respectively of the total linkage map. In addition to the linkage analysis, an association analysis will also be carried out on the data collected.

II. METHODS

A. TESTING OF CYSTIC FIBROSIS PATIENTS AND THEIR FAMILIES

Cystic fibrosis individuals and their families were contacted through three pulmonary and chest clinics in Lansing and Ann Arbor, Michigan and Cincinnati, Ohio. Contact was made with the director(s) of each clinic: Drs. Wortley and Honicky in Lansing, Drs. Kellogg and Ingberg in Cincinnati, and Drs. Howatt and Roloff in Ann Arbor. (Table 9)

With the permission of the clinic directors, cystic fibrosis individuals and accompanying family members were contacted at the time of their regular clinic visit. The majority of cystic fibrosis patients were children or teenagers, and most came to the clinic with

TABLE 8. MAP LOCATIONS OF TEST LOCI.[61]

	Test Locus or Loci	Map Location
1.	HLA system	6p2
2.	Rhesus	1p3
3.	Duffy	1q13
4.	ABO	9q3
5.	P	6q2
6.	MNSs	4q
7.	Lewis	unknown
8.	Secretor	unknown
9.	Kidd	7q
10.	Kell	unknown
11.	Alpha-1-antitrypsin	possibly 2 or 12

TABLE 9. CYSTIC FIBROSIS CLINICS.

1. Chest Clinic
   Drs. Wortley and Honicky
   Ingham Medical Center
   Lansing, Michigan

2. Chest Clinic
   Drs. Kellogg and Ingberg and Miss Lisa Richter
   Children's Hospital of Cincinnati
   Cincinnati, Ohio

3. Cystic Fibrosis Clinic
   Drs. Howatt and Roloff
   Mott Children's Hospital
   University of Michigan
   Ann Arbor, Michigan

one or both parents and frequently with siblings as well. The presence of a minimum of two family members, one affected individual and one relative (either parent or sibling) was the first prerequisite for contacting the family. If consent to participate in this study was obtained from two or more of the family members present at this clinic visit, then arrangements were made, either during the same visit or during a follow-up visit, to contact other family members.

Each participating individual was asked to sign a consent form. The consent form was approved for use by the committees on research in valuing human subjects of Michigan State University and the University of Michigan. Consent forms for all minors were co-signed by a parent or other accompanying adult. Any questions regarding the consent form were answered at the time of signing, and families were offered copies of the consent form to keep for their personal records.

Blood was obtained for typing the red and white blood cell antigens. A physician was present to supervise all venipunctures. Those venipunctures performed in the clinics were often performed by one of the directors or a member of the nursing staff at the clinic. A few families requested to be drawn at the histocompatibility lab at Michigan State University and simultaneously receive a brief tour of the research facilities; for these individuals, a physician was also present. A large number of families requested home visits to include additional family members, and again, a physician was present to supervise and/or perform the venipuncture.

Blood was drawn either directly into a heparin vacutainer tube, or was drawn into a syringe and immediately transferred to a heparin tube to prevent clot formation. The blood typing was initiated within twenty-four hours, the outside limit for lymphocyte viability for the histocompatibility antigen testing. Needles chosen for the venipuncture were 18 guage or larger, so that the cellular components of the blood would not be destroyed. All laboratory work was performed in the histocompatibility laboratory at Michigan State University.

B. TYPING OF THE HISTCOMPATIBILITY ANTIGENS

Typing of the human histocompatibility system was performed by the microcytotoxicity method.[64] Antigens tested were A1, A2, A3, A9 (A23 and A24), A10 (A25 and A26), A33, A34, A11, A28, A29, A30, A31, A32 and A25 at the HLA-A locus, B5, B7, B8, B12, B13, B14, B15, B16 (B38 and B39), B17, B18, B21, B22, B27, B35, B37 and B40 at the HLA-B locus, and C1, C2, C3 and C4 at the HLA-C locus. Each sample was tested against all available antisera. The HLA-C antisera were not available until the end of this study, thus many individuals were not typed for the HLA-C alleles.

Two lymphocyte separation proceedures were used. In the first proceedure, the heparinized sample was centrifuged for ten minutes at 500 g. The buffy coat was mixed with McCoy's medium and layered on Ficoll, then centrifuged twelve minutes at 500 g. The interface was transferred to a clean tube and centrifuged 1.5 minutes at 3000 g.

The resulting cell pellet was suspended in McCoy's medium and three drops of thrombin (100 NIH units per milliliter) was added to the tube (thrombin agglutinates platelets and granulocytes). The tubes were rotated two to five minutes, centrifuged two to three seconds at 1000 g, and the supernatant was transferred to a clean tube. The lymphocytes in the supernatant were then washed twice with McCoy's medium and adjusted to 2.5 - 5 x 10^6 cells per milliliter.[64]

Alternatively, one milliliter of whole blood was rotated with a pipette tip full of carbonyl iron for 15 to 30 minutes. The blood was mixed with four milliliters of GEPS solution (EDTA phosphate saline), layered over Ficoll and centrifuged for 30 minutes at 400 g. The interface was centrifuged for 10 minutes at 325 g and the cell pellet was washed twice with GEPS and twice with McCoy's medium. The lymphocytes in McCoy's medium were adjusted on the hemocytometer to 2.5 - 5 x 10^6 cells per milliliter.[64]

The lymphocytes were then dispensed in one microliter amounts onto prepared antisera trays to test for antigenic specificities. The trays each contained 60 to 72 wells (Terasaki or Michigan State University trays, respectively), and each well held a different antiserum. Each tray had two wells designated to act as positive and negative controls. Five microliters of complement was then mixed in each well and the trays were incubated for 60 minutes. Eosin was mixed in each well, the trays were incubated for 5 minutes, and then formalin was added to each well to halt the reaction. The trays were sealed under a glass coverslip with paraffin and read under an inverted microscope: 1 = negative, the same percentage of cells

living as in the negative control of McCoy's medium, 2 = doubtful negative, 4 = doubtful positive, 6 = positive, viability noticeably different from controls, 8 = strong positive, more than 90% of cells killed (comparable to the positive control), and 0 = not readable. When a tray did not result in a satisfactory typing, another tray was analyzed. Each individual was assigned two haplotypes, based on the typing results and the pedigree analysis. (Appendices A and B).

C. TYPING OF THE RED BLOOD CELL ANTIGENS

The ABO blood group was also typed and recorded at the time of the HLA typing. Red cell fractions from each sample were then sent to the American Red Cross Great Lakes Regional Blood Center of Lansing, Michigan for typing of the ABO, Rhesus, MNSs, Lewis-Secretor, P, Kell, Duffy and Kidd blood groups. The following red cell antigens were tested in all samples: A and B (ABO), D, C, E and c (Rhesus), M, N, S and s (MNSs), Le^a and Le^b (Lewis-Secretor), P_1 (P), Fy^a (Duffy) and Jk^a (Kidd). Some individuals were also tested for A_1 (ABO), D^u and e (Rhesus), k (Kidd) and Fy^b (Duffy) antigens. (Table 10) Each of the above loci contain a number of rare antigens or alleles as well as the common alleles listed above. Since the cost of typing these rare antigens would probably not have outweighed the information gained, these rare alleles were not typed.

TABLE 10. RED CELL ANTIGENS TYPED.

	System	Antigens
1.	ABO	A_1, A_2, B
2.	Rhesus	D, C, E, c, e, D^u
3.	MNSs	M, N, S, s
4.	Lewis-Secretor	Le^a, Le^b
5.	Kell	K, k
6.	P	P_1
7.	Duffy	Fy^a,Fy^b
8.	Kidd	Jk^a

D. TESTING OF α_1 ANTITRYPSIN

Alpha-1-antitrypsin was quantitatively determined by a starch-gel electrophoresis method. Between 1/4 to 2 milliliters of serum was extracted from each sample and stored at -4° C until electrophoresis was performed. Since qualitative studies were not performed, the specific genotype of any individual could not be determined by this method; however, this test would differentiate any individual with a deficiency of serum alpha-1-antitrypsin. Since the most common cause of a deficiency of serum alpha-1-antitrypsin is the presence of the Z allele, this test served as an inexpensive screen for the MZ genotype in cystic fibrosis family members.

E. LINKAGE ANALYSIS

In relation to the cystic fibrosis or main locus, all parents were assumed to be heterozygous. The mating type of the parents is thus Cfcf x Cfcf. It was assumed that no new mutations occurred at the cystic fibrosis locus. For the test loci, the mating type of each family at each test locus was determined from the pedigree and typing information available. For example, at the ABO locus, a type O child born to type A parents indicates a parental mating type of AO x AO. Mating types such as OO x OO or AA x BB give no linkage information because no segregation is visible. One parent only shows visible segregation in matings such as AO x OO or BO x AA, and both parents show segregation in such matings as AB x AB. A few matings could not be scored because of insufficient information such as if

both parents are type A and all tested children are type A: the mating type of the parents may have been AO x AO, however lacking the presence of an OO child or informative grandparents, the mating type remains unknown and yeilds no linkage information. Grandparents were typed when possible, to provide additional information on the parental genotypes.

For each family, lod scores were obtained for testing linkage between the cystic fibrosis locus and each test locus, at the following map distances (values of theta): 0.00, 0.05, 0.10, 0.20, 0.30 and 0.40 centimorgans. The scores of all families for each test locus and each map distance were then added and cumulative scores were obtained. Linkage is considered present if a total lod score of +3 is attained for all families tested, for any given map distance. The value +3 represents odds of 1000 to 1 that the conclusion of linkage is correct. Similarly, a lod score of -2 or less indicates that linkage is absent between 2 loci at a given map distance, with a probability of 100 to 1 of accuracy.

Lod scores for each family at a single test locus may be calculated in three ways. For example, at the HLA locus, the father and mother have haplotypes A, B and C, D, respectively. Their children may have cystic fibrosis and HLA genotypes Cf-A/C, cfcf A/C and cfcf A/D. First, a lod (z) score may be calculated for segregation of cystic fibrosis and HLA alleles from the father to the children. The z_2 formula is used (mating type 2, Tables 4 and 5). All three children received haplotype A from the father and two are affected. Thus, the

progeny phenotypes are a (CfT1), b (cfT1) and b (cfT1), respectively. No correction (e) score is needed since the father would express both haplotypes serologically. Second, a lod (z) score may be calculated for segregation of the cystic fibrosis and HLA alleles from the mother. Mating type 2 is used again, and progeny phenotypes are a (CfT1), b (cfT1) and d (cfT1T2), respectively. Third, since both parents show visible segregation, a "both" or combined score may be calculated using mating type 6 and z score formula z_7. Progeny phenotypes are a (CfT1T3), b (cfT1T3) and f (cfT1T4).

Some matings cannot be scored for both parents because segregation is visible in only one parent. An example is mating type AB x OO (ABO blood group). A z score may be calculated for segregation of the A and B alleles of the father, but not for segregation of the OO alleles of the mother.

Finally, the scores may be added or totalled. Male scores of all families with visible segregation of the alleles of the male may be totalled at each test locus. Similarly, all families at that test locus with visible segregation of alleles in the mother may be added for a cumulative female score. And third, a cumulative combined score is obtained by adding the combined z scores of all families with visible segregation of the alleles of both parents. And last, a "total" score for all families at that test locus may be obtained by adding the most informative score for each family (a score for both parents is more informative than either a female or

a male score). Any family not showing any visible segregation in either parent is scored as zero (for example, an OO x OO mating at the ABO locus).

III. RESULTS

A. PARTICIPATION BY CYSTIC FIBROSIS FAMILIES

A total of fifty-nine families participated in this study. Seventeen families were from the Lansing area and seen at the Lansing clinic; thirty-three families were seen at the Cincinnati clinic and nine families at the Ann Arbor clinic. None of the families are known to be related.

A total of 244 individuals were typed. Of these, 67 individuals had cystic fibrosis, 51 individuals were unaffected siblings and 4 were unaffected half-siblings. Eighty-nine parents, 32 grandparents and 1 great-grandparent were typed. Some family members such as young children and infants were not typed because of technical difficulties in obtaining adequate blood samples.

B. SEX RATIO

The sex ratio of cystic fibrosis and unaffected children is consistent with expected values for an autosomal recessive trait. Of the 118 children in the study, 67 were affected and 51 were unaffected. Thirty-five of the affected children were males and 32 were females; 30 of the unaffected children were males and 21 were females. (Chi-square = .5075, no significant difference.)

C. ANALYSIS OF DATA OF THE RED AND WHITE BLOOD CELL ANTIGENS

A total of fifteen different loci were tested: the A, B and C loci of the HLA system, the D, C and E loci of the Rhesus system, the MN and Ss loci, and the P, Kell, Kidd, Lewis, Secretor, ABO and Duffy loci. These fifteen loci represent ten separate linkage groups: the MNSs, HLA, P, Kell, Kidd, Lewis, Secretor, ABO, Rhesus and Duffy linkage groups. (Table 8)

1. The Histocompatibility Complex

HLA typing was performed on each individual by a microcytotoxicity method as described above. The HLA-A, HLA-B and HLA-C loci antigens possessed by each individual were then combined into two haplotypes based on the HLA antigens of other family members. For example, in family BO2, mother has HLA alleles A2, A24, B7 and B12. Father has alleles A1, A2, B8 and B14. Two children, BO23 and BO25 have alleles A2, B12 and B14. These children each received an A2/B12 haplotype from the mother, and a A2/B14 haplotype from the father. Child BO24 received an A2/B14 haplotype from the father and an A24/B7 haplotype from the mother. Children BO26, BO27 and BO28 share the haplotype A1/B8 with the father, and have respective A24/B7, A2/B12 and A2/B12 haplotypes in common with their mother.

Haplotypes of each individual are listed in Appendix A. Three families are listed by individual antigens rather than by haplotypes, since family evidence was insufficient to determine haplotype

grouping (families B09, A43 and A49). For example, in family A49, the mother has HLA alleles A2, A3 and B7. The affected son has alleles A2, A29, B12 and B15. Since the mother did not type for either B12 or B15, no discernable haplotype is shared by the mother and affected son; however, it may be assumed that his A2 allele came from the mother. The unaffected son has alleles A2, A3, B7 and B15. He may share either A2 or A3 with the mother, at the HLA-A locus. At the B locus, he may share B7 with the mother, or both he and his affected sibling may share B15 with the mother. It is not possible in any of these three individuals to identify the HLA haplotypes.

Occasionally, a family occurred with a discrepancy in HLA typing. In family B32, the child B325 appears to be a half-sibling to the affected child B321. The haplotype A2/B5 is transmitted from the mother, but the reported father B323 has neither the A series antigen A26 or the B series B27. Also, the MNSs and Duffy antigens present in the child support nonpaternity. The child B325 was therefore assumed to be a half-sibling in the data analysis and computation of lod scores for linkage.

One family (A16) appeared to have a recombinant individual in the HLA-A system. (Figure 1) Based on the grandmother A162, the father A164 must have haplotypes A2/B35 and A2/B7. Each child received one intact haplotype from the father: A163 has A2/B35 and A165 has A2/B7. Thus child A163 must have haplotype A30/B12 and child A165 has A30/B35 from the mother. If the haplotypes of the mother are A28/B12 and A30/B35 then child A163 is a recombinant; if haplotypes

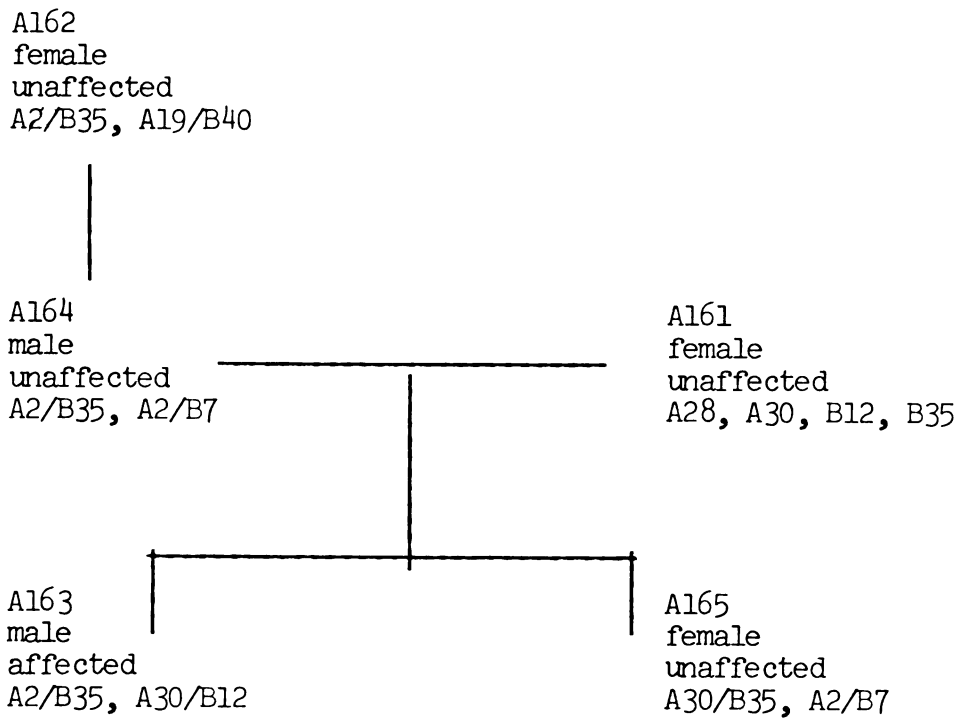

FIGURE 1. PEDIGREE OF FAMILY A16 ILLUSTRATING A RECOMBINANT EVENT AT THE HLA REGION.

of the mother are A28/B35 and A30/B12 then child A165 is a recombinant. The maternal grandparents were not available for typing.

Families B30 and A47 both have individuals demonstrating more than two alleles at a single locus. Individual B303 reacts positively to antisera for alleles B7, B12 and B40; individual A471 reacts to antisera for alleles A2, A25, and A32. Individual A471 passed these specificities to one child; in family B30 it is unclear if the double allele was passed from the mother or from the father. This type of reaction is probably the result of a typing error. Alternative explainations are due to an inserted allele (duplication) in the genome, or an allele which was combined with another allele (an intragenic recombinant event) so that the resulting molecule has receptor sites for antibodies of both type of alleles.

Last, individual C582 was not typed for HLA antigens because the blood sample obtained from her was of insufficient quantity.

All cystic fibrosis individuals and their full siblings were tested for any significant variation in the expected frequencies of each HLA antigen of both the A and B series alleles. (Table 11) Only one antigen, B7, manifested a highly significant difference (chi-square value between .0005 - .0010). Thirteen affected individuals out of a total of 67 carried this allele; 25 normal full siblings out of a total of 51 had the allele. The reason for this association of the B7 allele with unaffected siblings may be a chance occurrence or may be attributed to a relatively small sample size.

TABLE 11. CHI-SQUARE ANALYSIS OF ASSOCIATION OF WHITE BLOOD CELL ANTIGENS WITH CYSTIC FIBROSIS.

	Antigen Present		Antigen Absent			
Antigen	Affected	Not Affected	Affected	Not Affected	Chi-Square	Alpha
A1	15	13	52	38	0.1540	.6 - .7
A2	41	34	26	17	0.3744	.5 - .6
A3	13	13	54	38	0.6246	.4 - .5
A9	11	12	56	39	0.9333	.3 - .4
(A23)	4	1	63	50	1.1472	.2 - .3
(A24)	6	11	61	40	3.7361	.05 - .1
A10	6	4	61	47	0.0462	.8 - .9
(A25)	3	0	64	51	2.3432	.1 - .2
(A26)	1	3	66	48	1.7039	.1 - .2
A11	2	2	65	49	0.0775	.7 - .8
A28	6	5	61	46	0.0247	.8 - .9
A29	13	7	54	44	0.6631	.4 - .5
A30	3	2	64	49	0.0221	.8 - .9
A31	2	0	65	51	1.5486	.2 - .3
A32	8	5	59	46	0.1348	.7 - .8
A33	2	2	65	49	0.0775	.7 - .8
B5	7	7	60	44	0.2975	.5 - .6
B7	13	25	54	26	11.6339	.0005 - .0010
B8	8	10	59	41	1.3169	.2 - .3
B12	28	13	39	38	3.3937	.05 - .10
B13	5	2	62	49	0.6507	.4 - .5
B14	5	4	62	47	0.0059	.90 - .95
B15	13	6	54	45	1.2506	.2 - .3
B16	4	2	63	49	0.2518	.6 - .7
(B38)	1	1	66	50	0.0381	.8 - .9
(B39)	3	1	64	50	0.5601	.4 - .5
B17	4	1	63	50	1.1472	.2 - .3
B18	3	1	64	50	0.5601	.4 - .5
B21	3	5	64	46	1.2999	.2 - .3
B22	3	3	64	48	0.1184	.7 - .8
B27	4	6	63	45	1.2536	.2 - .3
B35	10	5	57	46	0.6845	.4 - .5
B37	4	0	63	51	3.1516	.05 - .10
B40	11	7	56	44	0.1624	.6 - .7

Example of calculation for the HLA-B7 Antigen:

d.f. = 1

$$\chi^2 = \frac{[(13 \times 26) - (54 \times 25)]^2 (118)}{(67)(51)(38)(80)} = 11.6339$$

α between .0010 - .0005

The histocompatibility complex was recognized by the haplotype derived from typing of the A, B and C loci and by correlation of the segregation of those alleles in each family. In 3 families, haplotypes could not be assigned to every individual because of insufficient information and lod scores were calculated based on the segregation of one locus instead of the entire haplotype. (Families B09, A43 and A49).

In family A16, (Figure 1), one of the two children received a recombinant haplotype from the mother, however information is not sufficient to determine which child is a recombinant and which received an intact haplotype from the mother. (The recombinant event took place between the A and B loci of the histocompatibility complex.) Although lod scores cannot be computed for cystic fibrosis and the HLA complex, lod scores can be computed for cystic fibrosis and a single HLA locus. Computing z_7 scores for this family using segregation of the A locus of the mother gives lod scores of +.1249, +.0859, +.0556, +.0184, +.0037 and +.0002 for map distances of 0, .05, .10, .20, .30 and .40 centimorgans, respectively. Calculation of z_7 scores using segregation of the HLA-B locus of the mother gives respective lod scores of +.1249, +.1219, +.1106, +.0780, +.0406 and +.0112. Both sets of scores are positive (give evidence for the presence of linkage). Lod scores of segregation of the HLA-A locus were arbitrarely chosen in this family for information of linkage with the cystic fibrosis locus.

Family B30 was not scored for linkage at the HLA locus. One child has both A series alleles in common with the mother and the other child has two B series alleles in common with the mother. (Appendix

A and B) The father and other siblings were not available and scoring of one child for A alleles and the other for B alleles would negate a recombinant event, if present.

All other families with two or more children were scored for linkage of the cystic fibrosis locus and the histocompatibility complex. (See Table 12 for lod scores, using the z_7 formula, Table 5). All families were scored for segregation of both parents using the z_7 formula. Each parent was also scored separately using the z_2 lod score formula. Linkage was excluded (total lod score value was less than -2) for 0.00, 0.05 and 0.10 centimorgans from the cystic fibrosis locus, using the cumulative lod scores of both parents for all families. (Figure 2) Linkage was excluded from 0.00 and 0.05 centimorgans when lod scores were calculated for segregation of the father; segregation of the mother excluded linkage of the HLA complex and cystic fibrosis from 0.00 centimorgans. One family could not be scored for the individual parents; family B22, two children were typed but consent was not obtained for the parents; since the parents were not typed, it is not known which parent had which haplotypes.

2. The ABO Blood Group

The ABO blood group antigens were typed twice, first at the histocompatibility lab at Michigan State University and then at the American Red Cross Great Lakes Regional Blood Center. For each individual the ABO type obtained from the two laboratories was the same. The ABO types were also consistent within each family (Appendix A). Results of the association analysis showed no significant

TABLE 12. LOD SCORES OF CYSTIC FIBROSIS AND THE HISTOCOMPATIBILITY COMPLEX.

			0.00	0.05	0.1	0.2	0.3	0.4
B01	z7	Both	-∞	-.6174	-.3597	-.1446	-.0532	-.0120
	z2	Female	-.0512	-.0410	-.0320	-.0177	-.0078	-.0019
	z2	Male	-.3522	-.2596	-.1908	-.0969	-.0404	-.0098
B02	z7	Both	-∞	-.4687	-.2522	-.0947	-.0347	-.0079
	z2	Female	-.2784	-.2186	-.1683	-.0910	-.0394	-.0096
	z2	Male	.0226	.0219	.0199	.0138	.0070	.0019
B03	z7	Both	.1249	.0859	.0556	.0184	.0037	.0002
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	z2	Male	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
A06	z7	Both	.4998	.4333	.3653	.2310	.1130	.0302
	z2	Female	.4998	.4354	.3703	.2407	.1210	.0335
	z2	Male	-.1023	-.0819	-.0641	-.0355	-.0156	-.0039
A14	z7	Both	-∞	-1.2399	-.7297	-.3110	-.1410	-.0729
	z2	Female	-.4033	-.3006	-.2229	-.1146	-.0482	-.0117
	z2	Male	-.4033	-.3006	-.2229	-.1146	-.0482	-.0117
A15	z7	Both	-∞	-.6174	-.3597	-.1446	-.0532	-.0120
	z2	Female	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
A16	z7	Both	.1249	.0859	.0556	.0184	.0037	.0002
	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B19	z7	Both	-∞	-.4965	-.2491	-.0666	-.0125	-.0008
	z2	Female	-∞	-.6838	-.4139	-.1768	-.0681	-.0158
	z2	Male	.2499	.2167	.1828	.1158	.0567	.0151
B21	z7	Both	.1249	.1210	.1106	.0780	.0406	.0112
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B22	z7	Both	.1249	.0859	.0556	.0184	.0037	.0002
B23	z7	Both	.7270	.6181	.5100	.3059	.1398	.0349
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	z2	Male	.4260	.3711	.3153	.2049	.1027	.0280
B24	z7	Both	-∞	-.9617	-.4907	-.1395	-.0282	-.0019
	z2	Female	.2725	.2482	.2192	.1509	.0793	.0222
	z2	Male	-∞	-.8712	-.5398	-.2470	-.0991	-.0235

TABLE 12 (Continued)

			0.00	0.05	0.1	0.2	0.3	0.4
A26	z7	Both	-∞	-.7622	-.4757	-.2116	-.0835	-.0197
	z2	Female	-.3522	-.2596	-.1908	-.0969	-.0404	-.0098
	z2	Male	-.3522	-.2596	-.1908	-.0969	-.0404	-.0098
B27	z7	Both	-∞	-.3490	-.1237	.0307	.0734	.0866
	z2	Female	.4260	.3711	.3153	.2041	.1027	.0280
	z2	Male	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
B31	z7	Both	.1249	.0859	.0556	.0184	.0037	.0002
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	z2	Male	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
B32	z7	Both	.1249	.1210	.1106	.0780	.0406	.0112
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B36	z7	Both	-∞	-1.0747	-.5775	-.1905	-.0539	-.0092
	z2	Female	-.0512	-.0492	-.0442	-.0295	-.0144	-.0038
	z2	Male	-.0512	-.0492	-.0442	-.0295	-.0144	-.0038
B37	z7	Both	.1249	.0859	.0556	.0184	.0037	.0002
	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B38	z7	Both	.3748	.3651	.3394	.2544	.1444	.0432
	z2	Female	.3748	.3231	.2715	.1717	.0843	.0226
	z2	Male	.3748	.3231	.2715	.1717	.0843	.0226
B39	z7	Both	.4998	.3935	.2949	.1373	.0461	.0087
	z2	Female	.1987	.1712	.1434	.0895	.0431	.0114
	z2	Male	-.1023	-.0819	-.0641	-.0355	-.0156	-.0039
B41	z7	Both	.1249	.1210	.1106	.0780	.0406	.0112
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B42	z7	Both	-∞	-.6174	-.3597	-.1446	-.0532	-.0120
	z2	Female	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
A47	z7	Both	-∞	-1.4425	-.8874	-.3876	-.1514	-.0355
	z2	Female	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
	z2	Male	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
A48	z7	Both	-∞	-.6174	-.3597	-.1446	-.0532	-.0120
	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	z2	Male	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058

TABLE 12 (Continued)

		0.00	0.05	0.1	0.2	0.3	0.4
TOTALS:	Female	$-\infty$	-1.9547	-.8921	-.1223	.0501	.0294
	Male	$-\infty$	-2.0971	-1.1392	-.3602	-.0876	-.0110
	Both	$-\infty$	-6.8341	-3.1054	-.7314	-.0610	.0423
	Total	$-\infty$	-6.8341	-3.1054	-.7314	-.0610	.0423

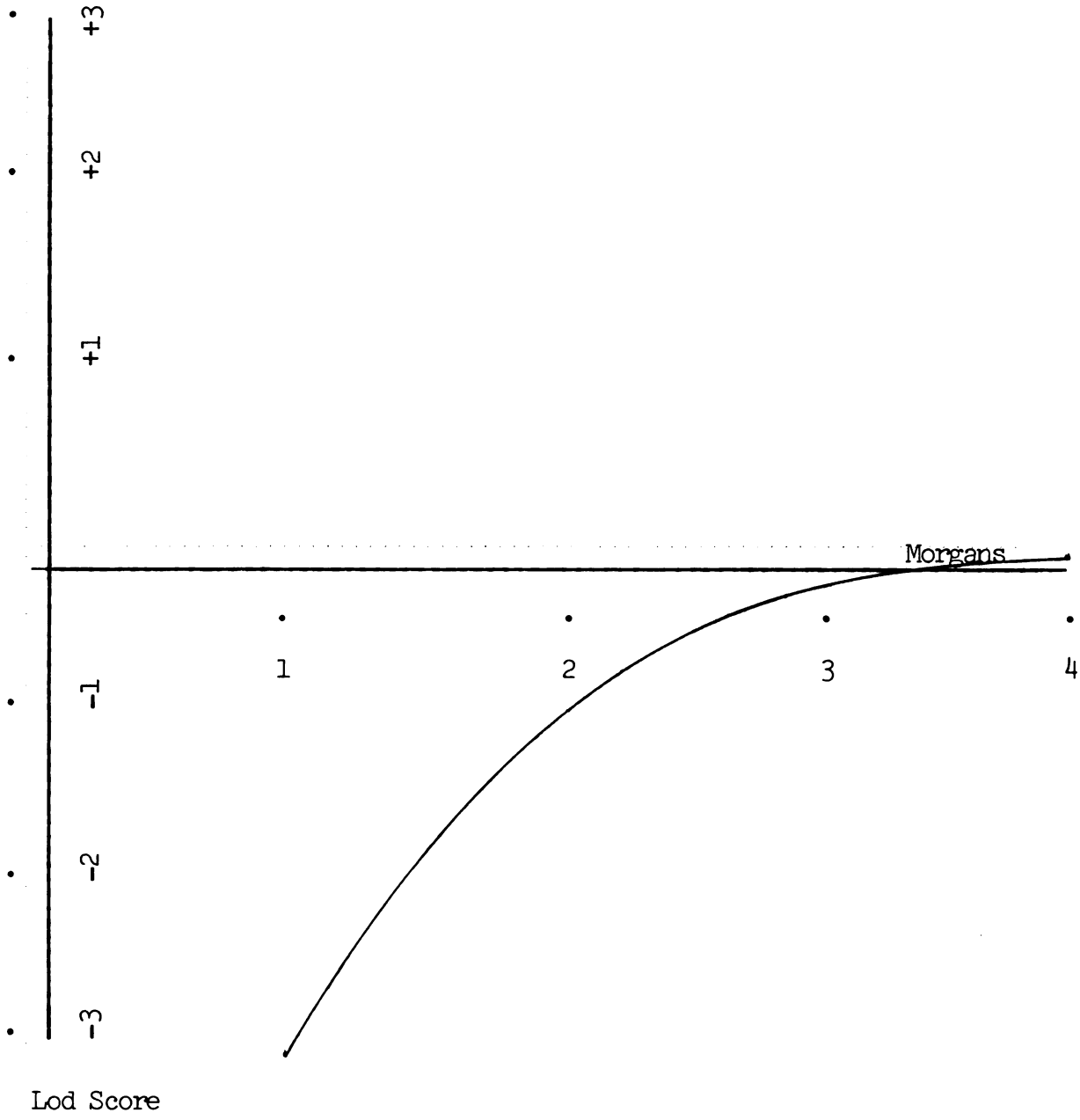

FIGURE 2. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE HISTOCOMPATIBILITY COMPLEX.

association of any of the ABO antigens with cystic fibrosis (Table 13). The combined lod scores of all families and those showing segregation of alleles by the father exclude linkage from 0.00 and 0.05 centimorgans. Segregation by the mother and those families where segregation could not be separated in the parents (both or combined scores) exclude linkage from 0.00 centimorgans. (Table 14, Figure 3).

3. The Rhesus Blood Group

Typing of the alleles of the Rhesus system yielded one discrepancy. In family A15, child A157 has alleles D, C, c and e. The parents A154 and A151 each have alleles D, C and e but do not have the c allele. Typing of all other antigen systems is consistent between parents and child; there are no conflicts in antigens present in two affected siblings. Since only one allele is unexplained, the pedigree is assumed to be accurate. The c allele in individual A157 may be the result of inaccurate typing, or a result of some other irregularity in genetic transmission or expression.

There is no significant association between possession of any of the Rhesus antigens and expression of cystic fibrosis. (Table 13).

Linkage between the Rhesus and cystic fibrosis loci is also negative. Lod scores exclude linkage at 0.00 and 0.05 centimorgans for all families; and from 0.00 map units for those families scored for segregation of male, female and combined alleles. (Table 15, Figure 4).

TABLE 13. CHI-SQUARE ANALYSIS OF ASSOCIATION OF RED BLOOD CELL ANTIGENS WITH CYSTIC FIBROSIS.

Trait	Antigen	Antigen Present		Antigen Absent		Chi-Square	Alpha
		Affected	Not Affected	Affected	Not Affected		
ABO	A	34	19	33	32	2.1303	.1-.2
	B	6	6	61	45	0.2502	.6-.7
	00	29	27	38	24	1.0831	.2-.3
	A_1	21	13	43	37	0.6224	.4-.5
Rhesus	D	56	39	11	11	0.5844	.4-.5
	C	45	27	22	23	2.0964	.1-.2
	E	19	13	48	37	0.0801	.7-.8
	c	52	42	15	8	0.7398	.3-.4
	e	65	47	2	3	0.6362	.4-.5
MNSs	M	50	44	17	6	3.2422	.05-.1
	N	46	31	21	19	0.5713	.4-.5
	S	29	30	38	20	3.2006	.05-.1
	s	58	42	9	8	0.1519	.6-.7
Lewis	Le(a-b-)	8	6	59	44	0.0001	.975-.990
	Le(a+b-)	14	6	53	44	1.5987	.2-.3
	Le(a-b+)	45	38	22	12	1.0843	.2-.3
Kell	K	7	6	60	44	0.0699	.7-.8
P	P_1	56	41	11	9	0.0506	.8-.9
Duffy	Fy^a	41	23	26	27	2.6676	.1-.2
	Fy^b	40	14	4	1	0.0848	.7-.8
Kidd	Jk^a	55	42	12	8	0.0737	.7-.8
Sex	Female	32	21	35	30	0.5075	.4-.5

TABLE 14. LOD SCORES OF CYSTIC FIBROSIS AND THE ABO BLOOD GROUP.

			0.0	0.05	0.1	0.2	0.3	0.4
B01	z2	Male	-.0512	-.0410	-.0320	-.0177	-.0078	-.0019
B02	z2	Female	.0226	.0219	.0199	.0138	.0070	.0019
	e2		-.0060	-.0045	-.0033	-.0017	-.0007	-.0002
A15	z3	Both	.2499	.2076	.1680	.0984	.0451	.0115
B19	z2	Male	.0738	.0628	.0519	.0315	.0148	.0038
	e2		-.0032	-.0031	-.0028	-.0019	-.0009	-.0003
B21	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
B23	z2	Male	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
	e2		.0212	.0171	.0134	.0075	.0033	.0008
A26	z7	Both	.2499	.2076	.1680	.0984	.0451	.0115
	e2		-.0332	-.0271	-.0215	-.0122	-.0055	-.0014
	z2	Female	-.0512	-.0410	-.0320	-.0177	-.0078	-.0019
	z2	Male	.2499	.2122	.1754	.1072	.0509	.0133
	e2		-.0332	-.0271	-.0215	-.0122	-.0055	-.0014
B31	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
B36	z2	Male	-∞	-.7622	-.4757	-.2115	-.0835	-.0197
	e2		-.0032	-.0031	-.0028	-.0019	-.0009	-.0003
B37	z7	Both	.1249	.1210	.1106	.0780	.0406	.0112
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B38	z2	Female	-.5283	-.3608	-.2532	-.1219	-.0494	-.0118
	e2		-.0199	-.0160	-.0126	-.0070	-.0031	-.0008
B39	z2	Male	-.4033	-.3006	-.2229	-.1146	-.0482	-.0117
	e2		-.0111	-.0087	-.0067	-.0036	-.0015	-.0004

TABLE 14. (Continued)

A47	z7	Both	-∞	-1.4425	-.8874	-.3876	-.1514	-.0355
	e3		.0631	.0443	.0305	.0132	.0047	.0010
	z2	Female	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
	e2		.0212	.0171	.0134	.0075	.0033	.0008
	z2	Male	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
	e2		.0212	.0171	.0134	.0075	.0033	.0008
TOTALS:		Female	-∞	-.9717	-.6031	-.2564	-.0966	-.0219
		Male	-∞	-2.1088	-1.2595	-.5159	-.1899	-.0427
		Both	-∞	-.9265	-.4616	-.1288	-.0291	-.0036
		Total	-∞	-2.9131	-1.7237	-.6872	-.2459	-.0541

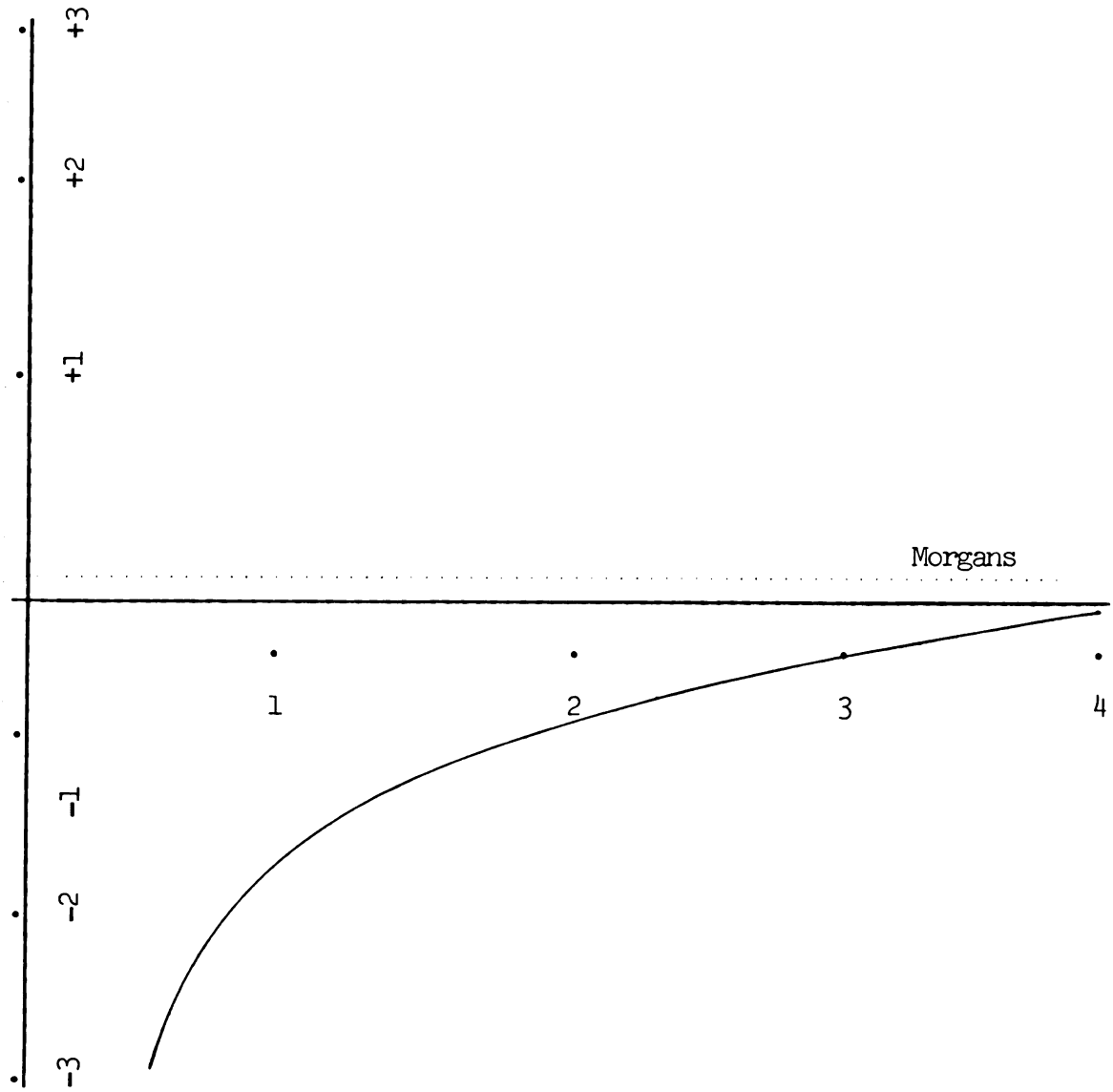

FIGURE 3. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE ABO BLOOD GROUP.

TABLE 15. LOD SCORES OF CYSTIC FIBROSIS AND THE RHESUS BLOOD GROUP.

			0.0	0.05	0.1	0.2	0.3	0.4
B01	z2	Female	-.0512	-.0410	-.0320	-.0177	-.0078	-.0019
B03	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
A06	z7	Both	.4998	.4873	.4547	.3471	.2036	.0635
	z2	Female	.4998	.4354	.3703	.2407	.1219	.0335
	z2	Male	.4998	.4354	.3703	.2407	.1219	.0335
A14	z2	Male	-.4033	-.3006	-.2229	-.1146	-.0482	-.0117
B19	z2	Male	-∞	-.7622	-.4757	-.2115	-.0835	-.0197
B21	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
B27	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B31	z7	Both	.1249	.1210	.1106	.0780	.0406	.0112
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B32	z7	Both	.1249	.0895	.0556	.0184	.0037	.0002
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	z2	Male	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
B36	z2	Male	-∞	-.7622	-.4757	-.2115	-.0835	-.0197
B37	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
B38	z7	Both	-∞	-.6666	-.4039	-.1741	-.0676	-.0158
	z2	Female	-.2272	-.1776	-.1362	-.0732	-.0316	-.0078
	z2	Male	-.2272	-.1776	-.1362	-.0732	-.0316	-.0078
B39	z4	Both	.1987	.1747	.1472	.0886	.0300	-.0218
	z2	Female	.7270	.1038	.0840	.0492	.0226	.0058
	z2	Male	.7270	.1038	.0840	.0492	.0226	.0058
B41	z7	Both	.1249	.0859	.0556	.0184	.0037	.0002
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
B42	z2	Female	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
A47	z7	Both	-∞	-.3502	-.1284	.0103	.0296	.0866
	z2	Female	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
	z2	Male	.4260	.3711	.3153	.2041	.1027	.0280
A48	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058

TABLE 15. Continued)

		0.0	0.05	0.1	0.2	0.3	0.4
TOTALS:	Female	$-\infty$	-.9800	-.4737	-.1028	-.0035	.0058
	Male	$-\infty$	-.9176	-.3931	-.0247	.0444	.0200
	Both	$-\infty$	-.6045	-.0308	.2510	.1910	.1046
	Total	$-\infty$	-3.1501	-1.6372	-.4615	-.0875	.0397

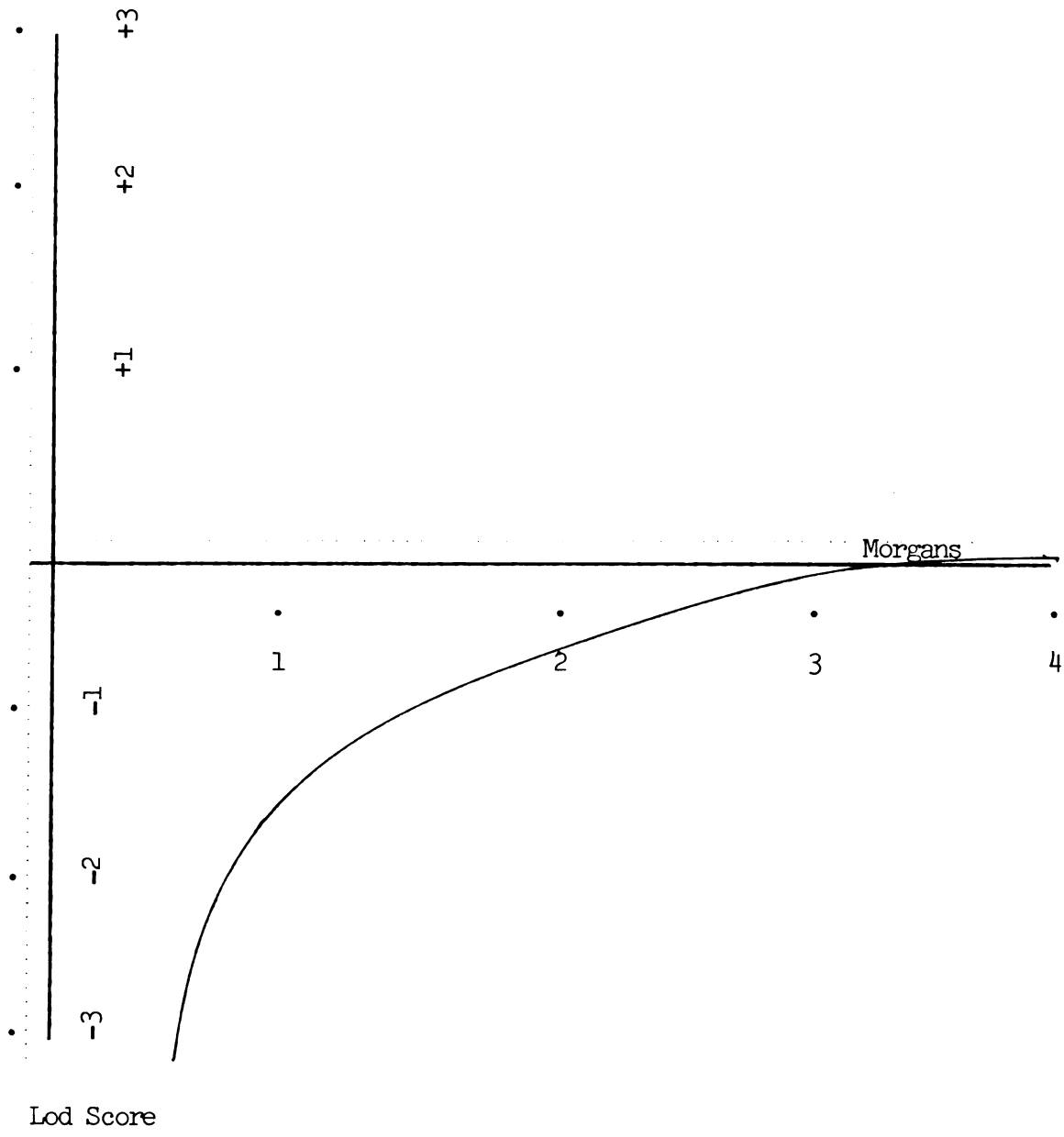

FIGURE 4. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE RHESUS BLOOD GROUP.

4. The MNSs Blood Group

The MNSs blood group shows no significant association of any single antigen with the cystic fibrosis locus.

Linkage between the MNSs and cystic fibrosis loci was excluded at 0.00 centimorgans for all four categories: families in which segregation is visible in males only, families in which segregation is visible in females, families in which segregation is visible in both parents, and all families. (Table 16, Figure 5).

5. The Lewis and Secretor Blood Groups

There was no significant association evident between the expression of Lewis-Secretor phenotype and cystic fibrosis (Table 13).

Linkage with cystic fibrosis was tested separately between the Lewis and the Secretor loci (Tables 17 and 18). For the Lewis and cystic fibrosis loci, linkage was excluded from 0.00 map distance for families with segregation visible in the female and for all families. Linkage was inconclusive for families with segregation visible in the male and for families with segregation visible in both parents. (Figure 6).

At the secretor locus, lod scores excluded linkage at 0.00 centimorgans for only one family with visible segregation in both parents. Linkage was inconclusive when scored for male and for female segregation. (Figure 7).

TABLE 16. LOD SCORES OF CYSTIC FIBROSIS AND THE MNSs BLOOD GROUP.

			0.0	0.05	0.1	0.2	0.3	0.4
B01	z7	Both	.2499	.1866	.1279	.0297	-.0354	-.0638
	z2	Female	-.0512	-.0410	-.0320	-.0177	-.0078	-.0019
	z2	Male	.2499	.2122	.1754	.1072	.0509	.0133
B02	z2	Female	-.2784	-.2186	-.1683	-.0910	-.0394	-.0096
A14	z7	Both	.4998	.3935	.2949	.1373	.0461	.0087
	z2	Female	.1987	.1712	.1434	.0895	.0431	.0114
	z2	Male	-.1023	-.0819	-.0641	-.0355	-.0156	-.0039
A15	z7	Both	-∞	-1.4425	-.8874	-.3876	-.1514	-.0355
	z2	Female	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
	z2	Male	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
B19	z4	Both	-∞	.1774	.3922	.5100	.4770	.3726
	z2	Female	.2499	.2122	.1754	.1072	.0509	.0133
	z2	Male	.2499	.2122	.1754	.1072	.0509	.0133
B21	z4	Both	.1249	.1079	.0987	.0962	.1015	.1036
B23	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
B24	z2	Male	-.6306	-.5332	-.4205	-.2264	-.0962	-.0234
A26	z4	Both	-.0512	-.0347	-.0266	-.0257	-.0353	-.0477
B27	z2	Female	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
B30	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B31	z4	Both	.1249	.1079	.0987	.0962	.1015	.1036
B32	z2	Male	.1249	..1038	.0840	.0492	.0226	.0058
B36	z4	Both	-∞	-.7246	-.4235	-.1638	-.0569	-.0122
	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	z2	Male	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
B37	z7	Both	.1249	.0859	.0556	.0184	.0037	.0002
	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B38	z2	Female	.0738	.0628	.0519	.0315	.0148	.0038
B41	z7	Both	.1249	.1210	.1106	.0780	..0406	.0112
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058

TABLE 16. (Continued)

B42	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
A47	z2	Female	.4260	.3711	.3153	.2041	.1027	.0280
TOTALS		Female	-∞	-1.1910	-.6303	-.1813	-.0859	-.0020
		Male	-∞	-.5296	-.2617	-.0508	.0035	.0048
		Both	-∞	-1.0216	-.1589	.3887	.4914	.4407
		Total	-∞	-1.8860	-.6764	.2052	.3916	.4334

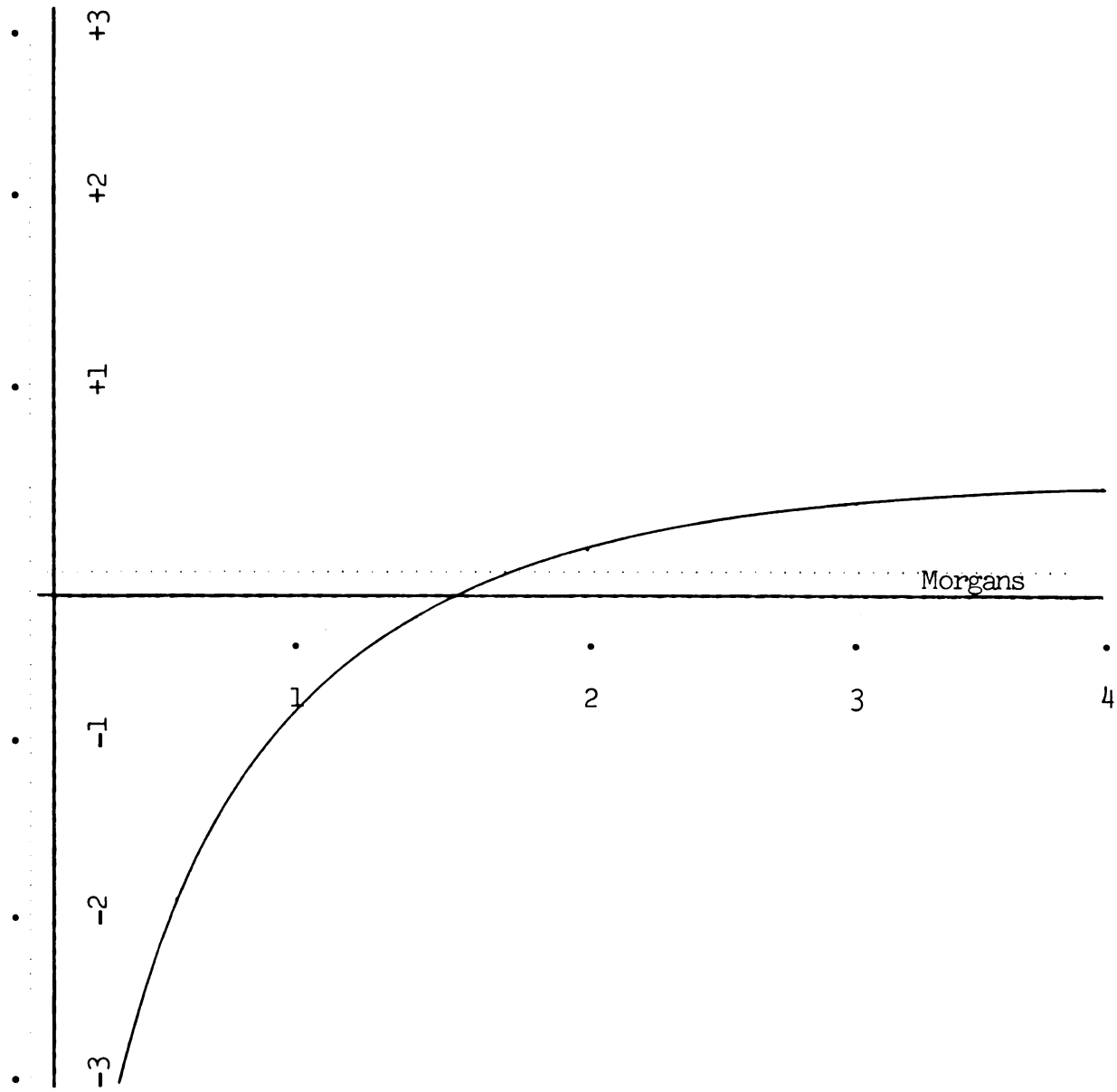

FIGURE 5. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE MNSs BLOOD GROUP.

TABLE 17. LOD SCORES OF CYSTIC FIBROSIS AND THE LEWIS BLOOD GROUP.

			0.0	0.05	0.1	0.2	0.3	0.4
B01	z2	Male	.2499	.2122	.1754	.1072	.0509	.0133
	e2		-.0332	-.0271	-.0215	-.0122	-.0055	-.0014
B02	z3	Both	.2725	.2296	.1882	.1123	.0518	.0132
	e3		-.0400	-.0275	-.0184	-.0075	-.0025	-.0005
	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	e2		.0212	.0171	.0134	.0075	.0033	.0008
	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
	e2		.0212	.0171	.0134	.0075	.0033	.0008
B19	z2	Female	-∞	-.6838	-.4139	-.1768	-.0681	-.0158
	e2		-.0032	-.0031	-.0028	-.0019	-.0009	-.0003
B23	z2	Male	.4260	.3711	.3153	.2041	.1027	.0280
	e2		.0212	.0171	.0134	.0075	.0033	.0008
TOTALS:		Female	-∞	-.5660	-.3193	-.1220	-.0431	-.0095
		Male	.8100	.6942	..5800	.3633	.1773	.0473
		Both	.2325	.2021	.1698	.1048	.0493	.0127
		Total	-∞	.0885	.2357	.2327	.1317	.0373

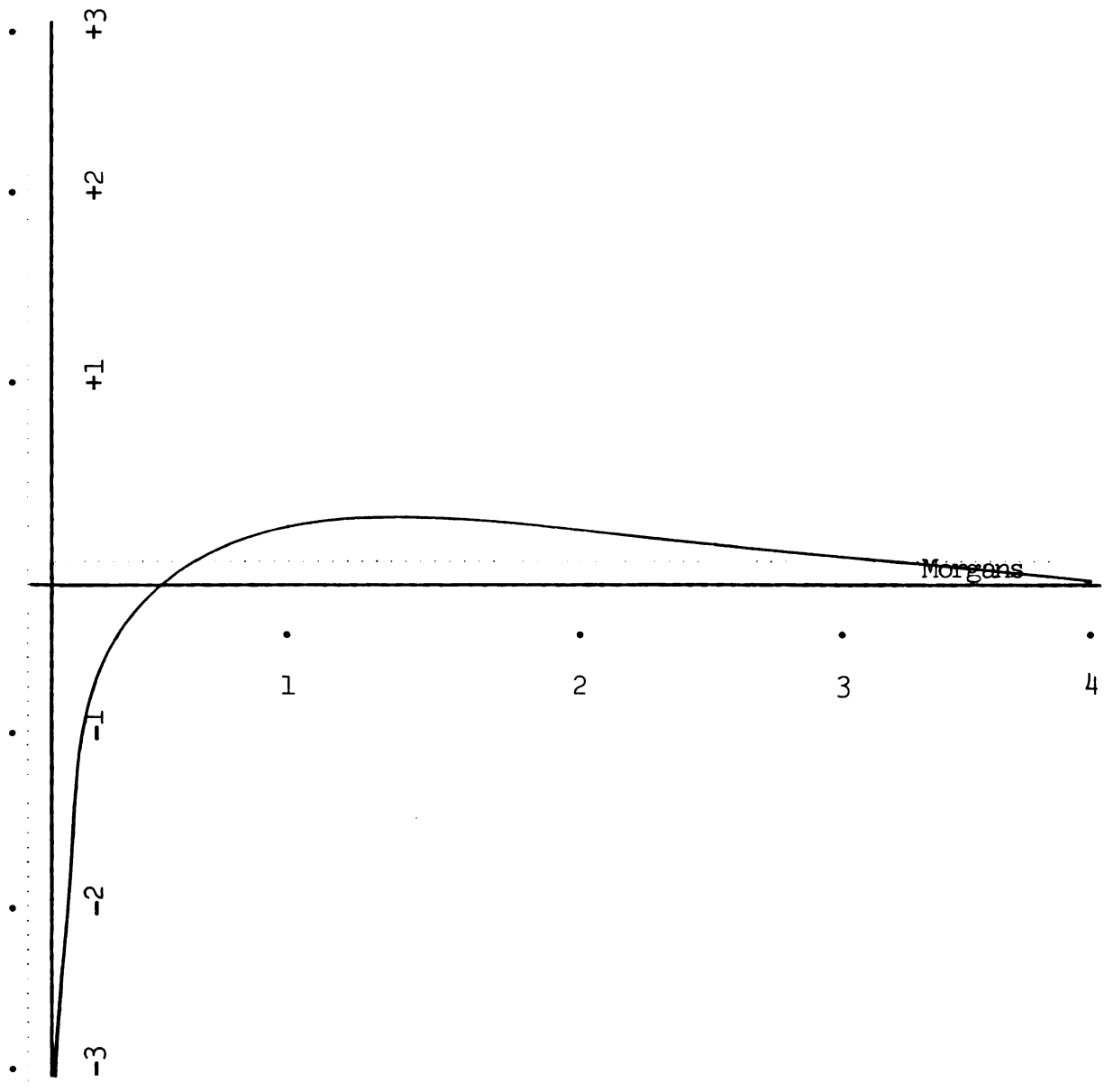

FIGURE 6. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE LEWIS BLOOD GROUP.

TABLE 18. LOD SCORE OF CYSTIC FIBROSIS AND THE SECRETOR BLOOD GROUP.

			0.0	0.05	0.1	0.2	0.3	0.4
B36	z3	Both	$-\infty$	-.7877	-.4859	-.2092	-.0801	-.0185
	e2		-.0032	-.0031	-.0028	-.0019	-.0009	-.0003
	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	z2	Male	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
TOTALS:		Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
		Male	-.2219	-.1741	-.1340	-.0725	-.0315	-.0077
		Both	$-\infty$	-.7908	-.4887	-.2111	-.0810	-.0188
		Total	$-\infty$	-.7908	-.4887	-.2111	-.0810	-.0188

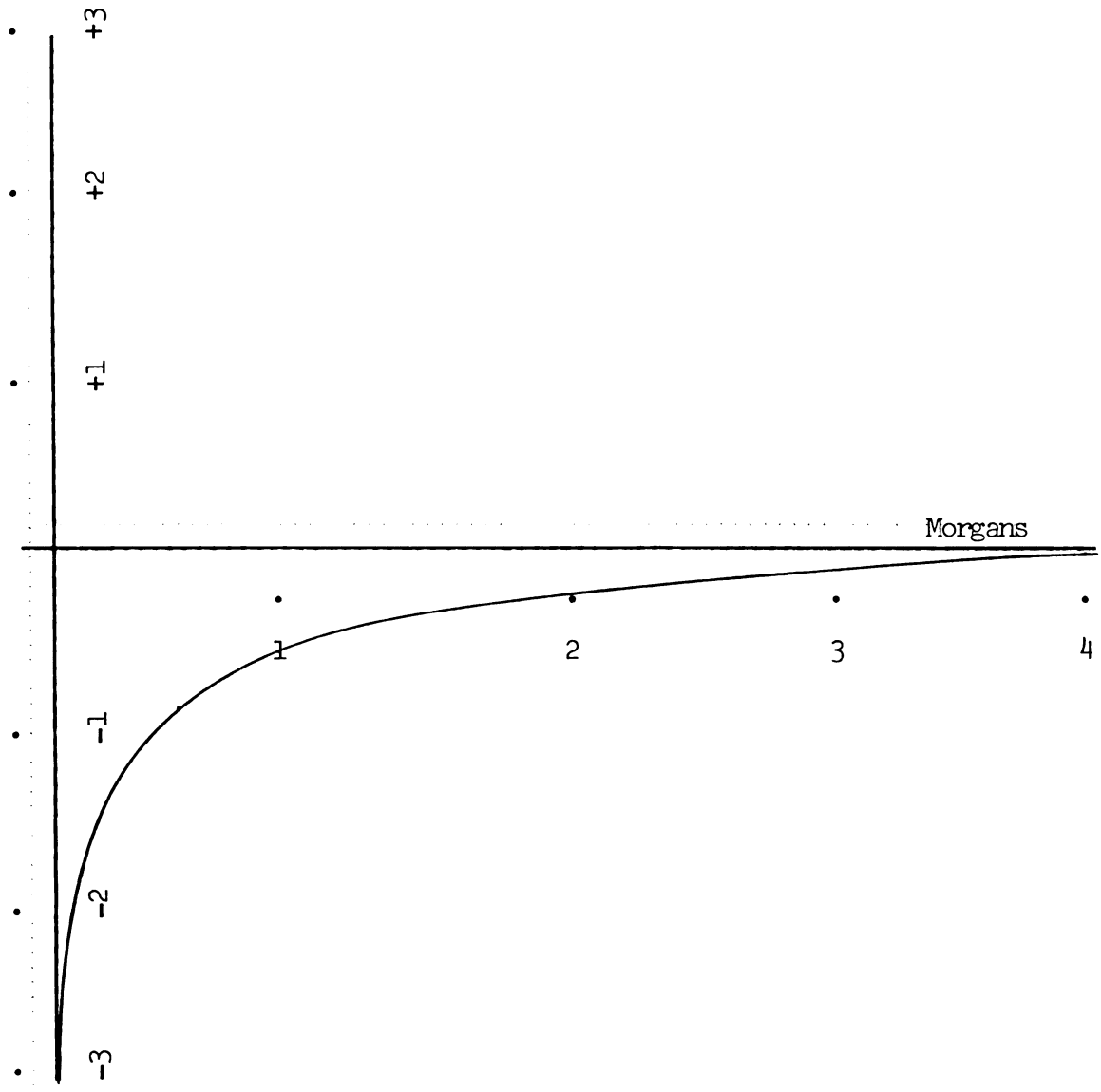

FIGURE 7: LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE SECRETOR BLOOD GROUP.

6. The Kell, P, Duffy and Kidd Blood Groups

There is no significant association of antigens of the Kell, P, Duffy or Kidd loci with the cystic fibrosis locus (Table 13).

Linkage at the Kell locus was inconclusive (greater than -2 and less than +3) for all categories: male scores, female scores, both or combined scores and total scores for all families (Table 19, Figure 8).

Linkage of the P blood group locus and the cystic fibrosis locus was excluded at 0.00 map units by female, both and total scores. Linkage was inconclusive based on the scores of the families segregating visibly in the fathers (Table 20, Figure 9).

At the Duffy and Kidd loci linkage was excluded at 0.00 map units for families with segregation by the fathers and for all families; scores for the females and the families with segregation at both loci was inconclusive (Table 21 and 22, Figures 10 and 11).

One individual (A165) was drawn at the completion of the study and was not typed for the red cell blood group antigens by the American Red Cross Great Lakes Regional Blood Center. However, she was typed for the histocompatibility antigens and for the ABO blood group alleles. Since only one other sibling in her family was included in this study, the family (A16) was scored in the linkage analysis for only the ABO and HLA loci. She was included only in the association analysis for ABO and HLA antigens and for sex distribution.

TABLE 19. LOD SCORES OF CYSTIC FIBROSIS AND THE KELL BLOOD GROUP.

			0.0	0.05	0.1	0.2	0.3	0.4
A06	z2	Male	.1987	.1712	.1434	.0895	.0431	.0114
A15	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B24	z2	Male	.8746	.7853	.6911	.4889	.2756	.0847
B30	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
B31	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
B37	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
B39	z2	Female	.4998	.4354	.3703	.2407	.1219	.0335
TOTALS:		Female	.7496	.6430	.5383	.3391	.1671	.0451
		Male	1.3231	1.1641	1.0025	.6768	.3639	.1077
		Both	0.0	0.0	0.0	0.0	0.0	0.0
		Total	2.0727	1.8071	1.5408	1.0159	.5310	.1528

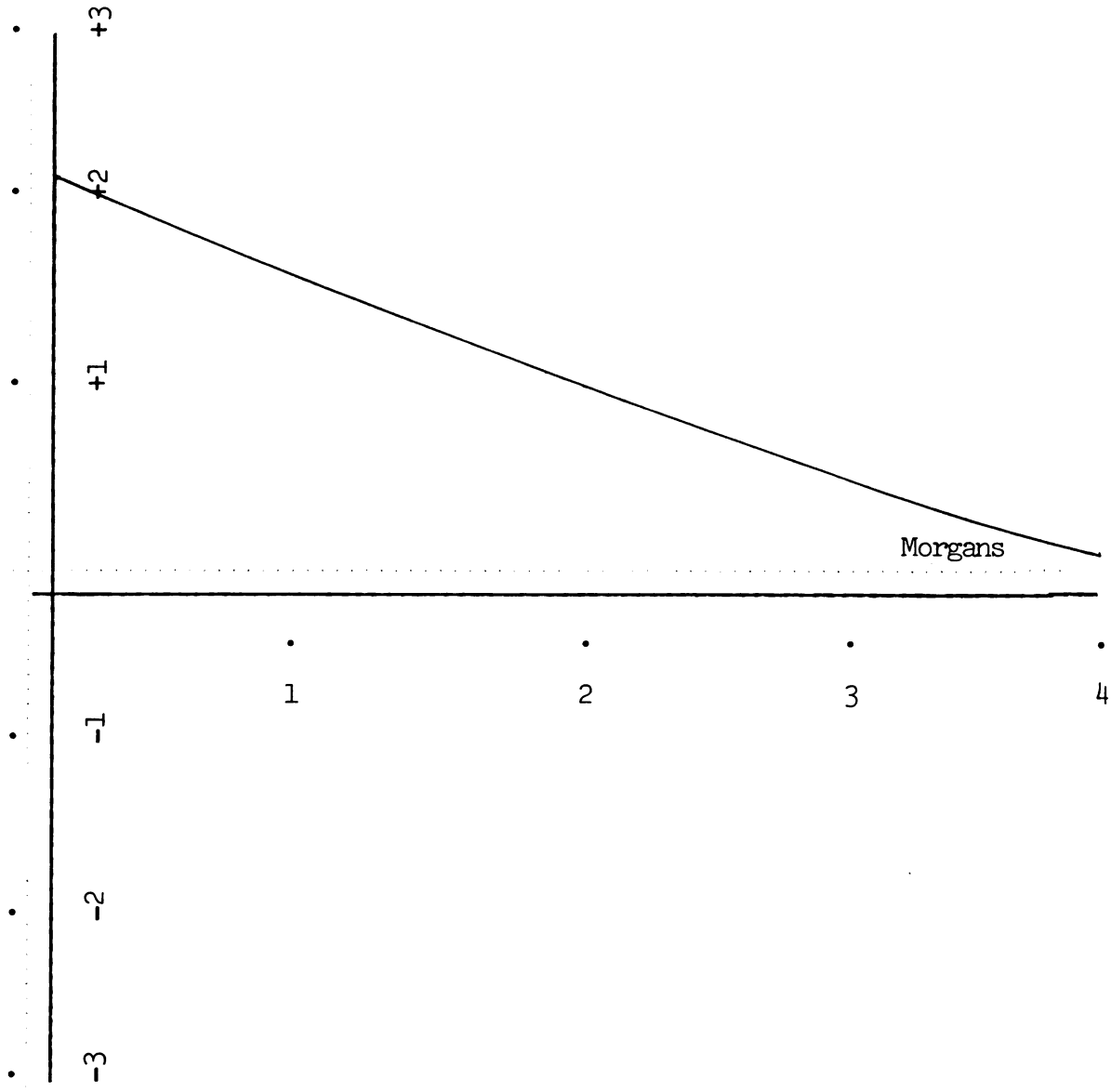

FIGURE 8. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE KELL BLOOD GROUP.

TABLE 20. LOD SCORES OF CYSTIC FIBROSIS AND THE P BLOOD GROUP.

			0.0	0.05	0.1	0.2	0.3	0.4
B01	z3	Both	.2499	.2076	.1680	.0984	.0451	.0115
	e3		-.0619	-.0470	-.0348	-.0173	-.0069	-.0016
	z2	Female	.0457	.0374	.0298	.0170	.0077	.0019
	e2		.0162	.0132	.0104	.0058	.0026	.0006
	z2	Male	.0457	.0374	.0298	.0170	.0077	.0019
	e2		.0162	.0132	.0104	.0058	.0026	.0006
B02	z2	Male	-.5794	-.4018	-.2853	-.1397	-.0572	-.0137
	e2		-.0060	-.0045	-.0033	-.0017	-.0007	-.0002
A15	z3	Both	-∞	-.5782	-.3270	-.1244	-.0435	-.0094
A26	z3	Both	.2499	.1993	.1543	.0824	.0346	.0083
	e3		-.0332	-.0271	-.0215	-.0122	-.0055	-.0014
B31	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
B32	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
B36	z3	Both	.1987	.1632	.1298	.0727	.0317	.0078
	e2		-.0032	-.0031	-.0028	-.0019	-.0009	-.0003
B37	z2	Male	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
B41	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
B42	z2	Female	-∞	-.7212	-.4437	-.1039	-.0757	-.0177
	e2		.0212	.0171	.0134	.0075	.0033	.0008
A48	z2	Male	.1249	.1038	.0840	.0492	.0226	.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
A49	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
TOTALS:		Female	-∞	-.9353	-.6039	-.1864	-.1102	-.0259
		Male	-.5872	-.3970	-.2740	-.1267	-.0493	-.0113
		Both	-∞	-.0853	.0660	.0977	.0546	.0149
		Total	-∞	-1.5188	-.8923	-.3594	-.1255	-.0196

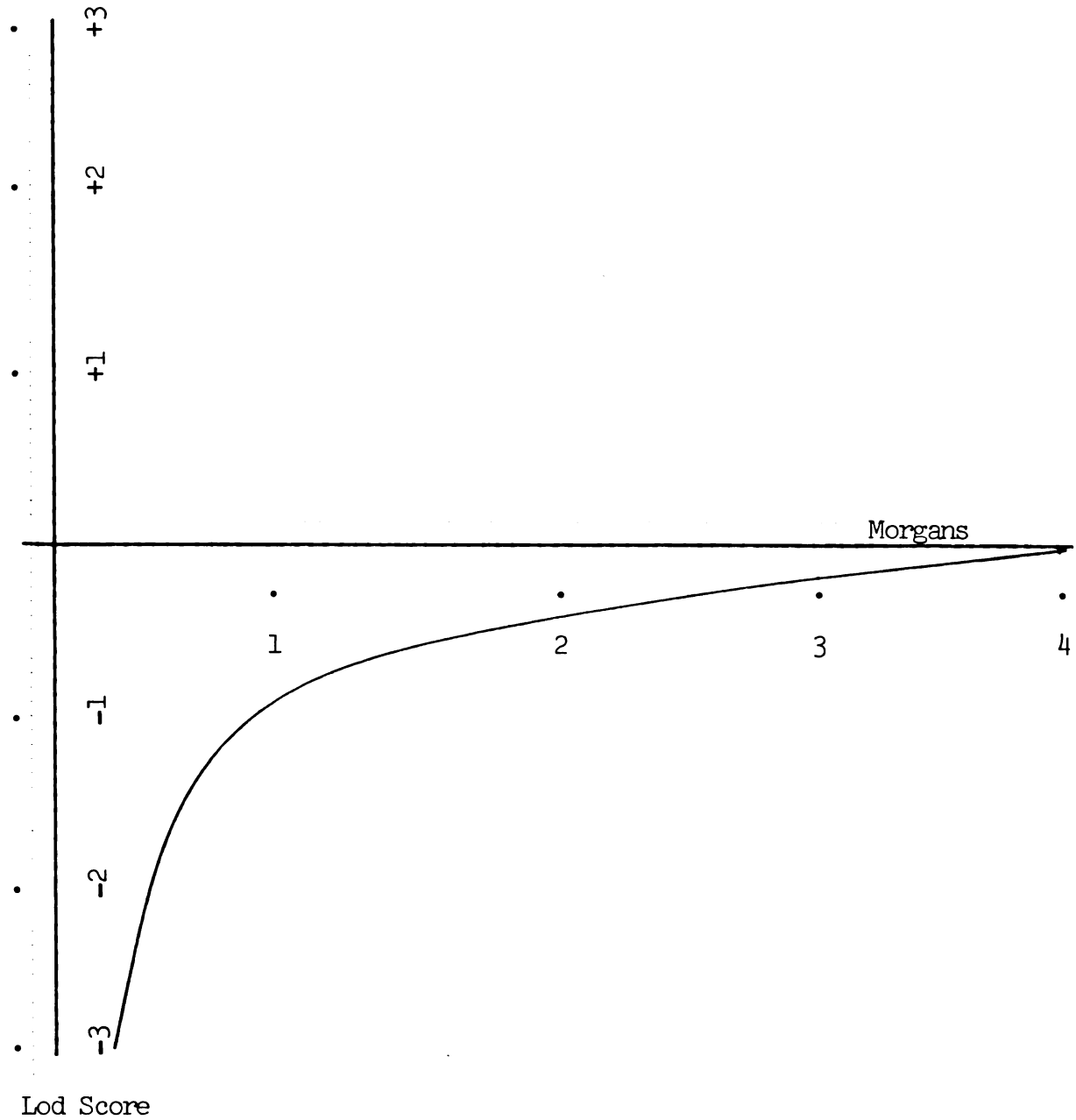

FIGURE 9. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE P BLOOD GROUP.

TABLE 21. LOD SCORES OF CYSTIC FIBROSIS AND THE DUFFY BLOOD GROUP.

			0.0	0.05	0.1	0.2	0.3	0.4
B02	z2	Female	-.5794	-.4018	-.2853	-.1397	-.0572	-.0137
	e2	..	-.0060	-.0045	-.0033	-.0017	-.0007	-.0002
A06	z2	Female	-.1023	-.0819	-.0641	-.0355	-.0156	-.0039
	e2		-.0111	-.0087	-.0067	-.0036	-.0015	-.0004
A14	z2	Female	.1987	.1712	.1434	.0895	.0431	.0114
	e2		-.0111	-.0087	-.0067	-.0036	-.0015	-.0004
A15	z6	Both	.7270	.6288	.5301	.3377	.1671	.0450
	z2	Female	.3010	.2577	.2148	.1335	.0645	.0170
	z2	Male	.3010	.2577	.2148	.1335	.0645	.0170
B19	z2	Male	-∞	-.7622	-.4757	-.2115	-.0835	-.0197
B21	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
A26	z2	Male	-.0512	-.0410	-.0320	-.0177	-.0078	-.0019
	e2		-.0332	-.0271	-.0215	-.0122	-.0055	-.0014
B31	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
B37	z2	Female	.1249	.1038	.0840	.0492	.0226	.0058
B39	z2	Male	-.4033	-.3006	-.2229	-.1146	-.0482	-.0117
TOTALS:		Female	-.1365	.0153	.0559	.0818	.0525	.0136
		Male	-∞	-.8732	-.5373	-.2225	-.0805	-.0177
		Both	.7270	.6288	.5301	.3377	.1671	.0450
		Total	-∞	-.7445	-.3809	-.0700	.0101	.0089

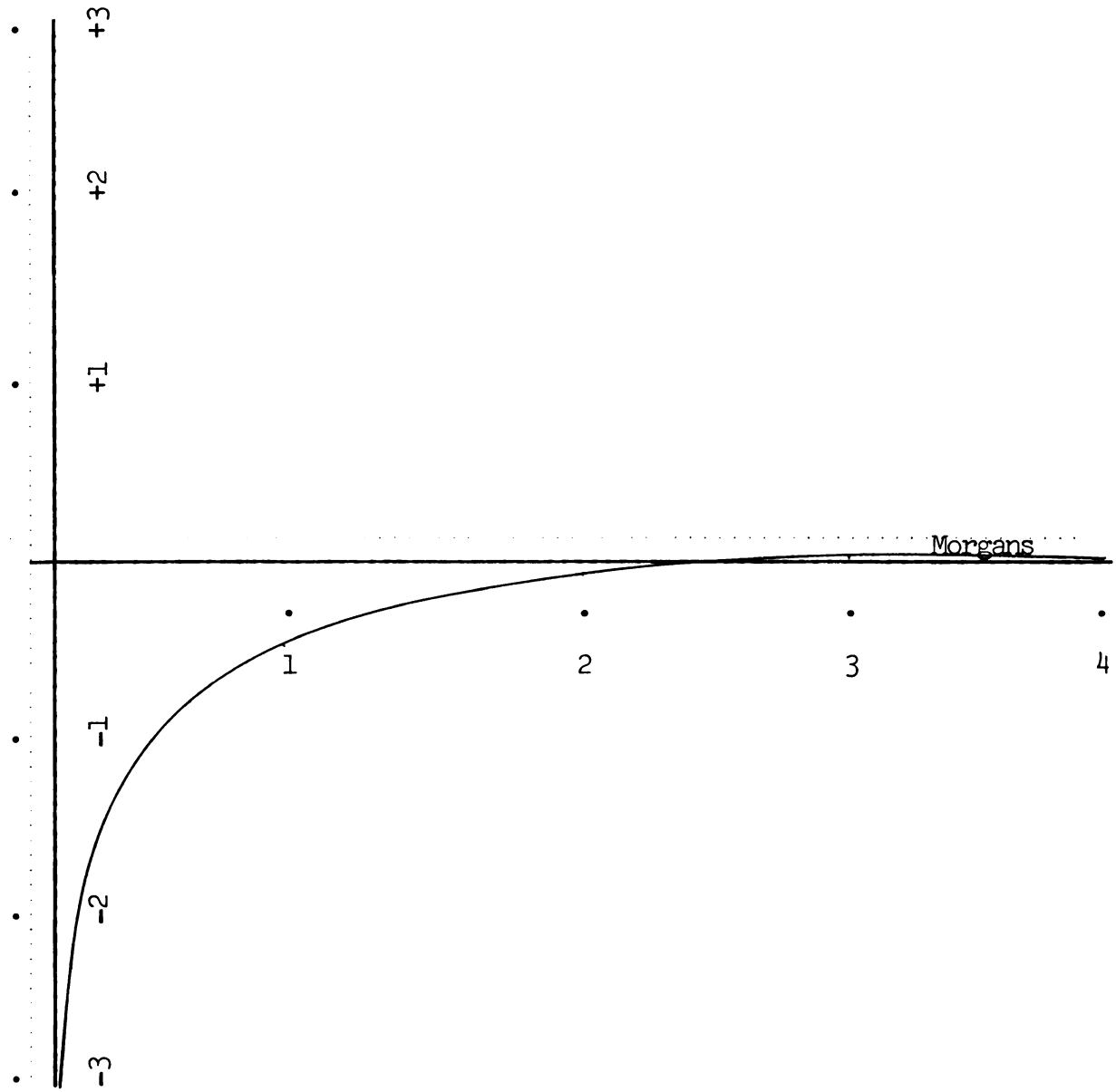

FIGURE 10. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE DUFFY BLOOD GROUP.

TABLE 22. LOD SCORES OF CYSTIC FIBROSIS AND THE KIDD BLOOD GROUP.

			0.0	0.05	0.1	0.2	0.3	0.4
B02	z3	Both	.6247	.5165	.4135	.2327	.1002	.0240
	e3		-.0400	-.0275	-.0184	-.0075	-.0025	-.0005
A06	z3	Both	-.0285	-.0322	-.0307	-.0208	-.0100	-.0026
	e3		-.0480	-.0345	-.0242	-.0108	-.0039	-.0008
A14	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
B23	z2	Female	.3010	.2577	.2148	.1335	.0645	.0170
	e2		.1761	.1367	.1042	.0555	.0238	.0058
B27	z2	Male	-∞	-.7212	-.4437	-.1938	-.0757	-.0177
	e2		.0212	.0171	.0134	.0075	.0033	.0008
B32	z2	Male	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
B39	z2	Male	-.4033	-.3006	-.2229	-.1146	-.0482	-.0117
	e2		-.0111	-.0087	-.0067	-.0036	-.0015	-.0004
A48	z2	Female	-.1761	-.1367	-.1042	-.0555	-.0238	-.0058
	e2		-.0458	-.0374	-.0298	-.0170	-.0077	-.0019
TOTALS:		Female	.0333	.0462	.0510	.0440	.0253	.0074
		Male	-∞	-.7931	-.4749	-.1880	-.0653	-.0139
		Both	.5082	.4223	.3402	.1936	.0838	.0201
		Total	-∞	-.7190	-.4027	-.1394	-.0445	-.0092

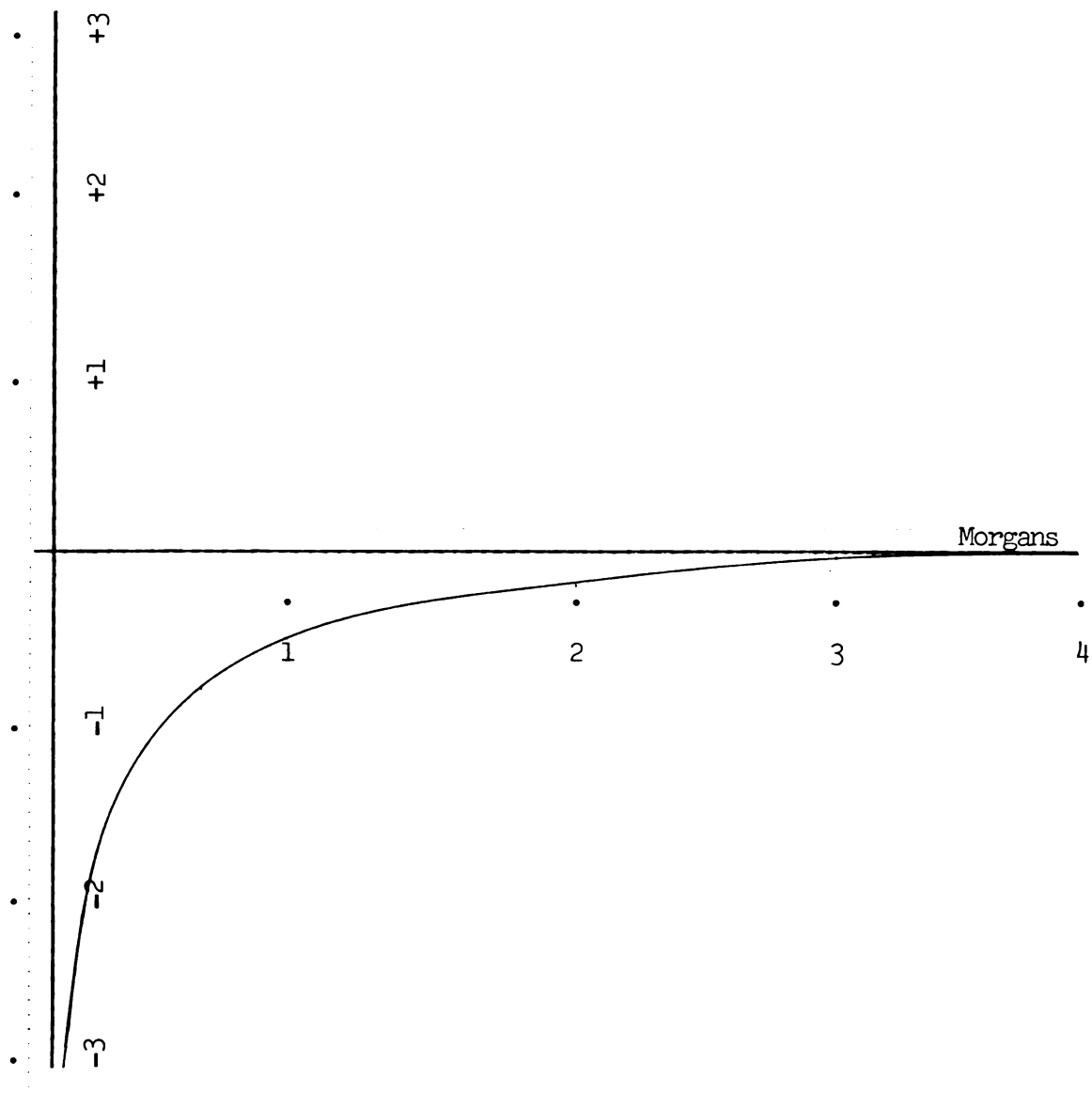

FIGURE 11. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE KIDD BLOOD GROUP.

D. RESULTS OF α_1 ANTITRYPSIN TESTING

Alpha-1-antitrypsin was quantitatively determined in each individual to show the presence of any of the deficiency alleles at the alpha-1-antitrypsin locus. Only two individuals exhibited a decreased amount of alpha-1-antitrypsin. Individuals A154 and A162 both had half the normal amount of alpha-1-antitrypsin in their serum. Individual A154 is the father of two cystic fibrosis children; individual A162 is a paternal grandmother of one cystic fibrosis child. These two individuals probably have one normal (M) and one deficiency (Z) allele at the alpha-1-antitrypsin locus. All other individuals in this study had normal amounts of alpha-1-antitrypsin.

IV. DISCUSSION

The ideal approach to diagnosis and treatment of cystic fibrosis depends on the ascertainment of the basic biochemical defect causing the disease. However, this biochemical alteration has continued to elude investigators and remains unknown. Since cystic fibrosis individuals do not yet benefit from the specific treatment given other genetic diseases, such as phenylketonuria, another approach must be sought.

A. ASSOCIATION OF BLOOD GROUP ANTIGENS AND CYSTIC FIBROSIS

Understanding of other diseases has benefited from association analyses, such as the association of the HLA-B27 allele of the histocompatibility complex with ankylosing spondylitis. In a general Caucasian population,

the B27 antigen occurs in about 5% of the individuals; the B27 antigen occurs in as many as 95% of patients having ankylosing spondylitis.[26, 65] This association is so strong that determination of the B27 antigen is now used as a diagnostic tool to differentiate between the many causes of lower back pain.

This study found no significant association of cystic fibrosis and HLA-B27. Four affected children and seven unaffected siblings were HLA-B27 positive (χ^2 value of 2.0604, alpha between .2 and .1).

A statistically significant increase in the frequency of the HLA-B18 allele of the histocompatibility complex has been found in cystic fibrosis individuals,[56] by Kaiser, Laszlo and Gyurkovits. Another study of 28 patients and 240 unrelated blood donors, by Gotz, Ludwig and Polymenidis, found no significant association of any HLA allele with cystic fibrosis.[57, 58] In this study, three affected individuals and one normal sibling carried the B18 allele and the difference is not significant.

However, a highly significant association was found between cystic fibrosis and the HLA-B7 allele. Thirteen affected individuals out of a total of 67 carried this allele, while 25 normal siblings out of a total of 51 had the allele (probability between .0010 and .0005). (Table 23)..

The conflicting results of these three studies may be due to statistical error present in small samples. Alternatively, these results may be explained if "cystic fibrosis" is composed of more

TABLE 23. ASSOCIATION OF HLA-B7 WITH CYSTIC FIBROSIS.

	HLA-B7 Positive	HLA-B7 Negative	Totals
Affected	13	54	67
Non-affected	25	26	51
Totals	38	80	118

$$\chi^2 = \frac{[(13 \times 26) - (54 \times 25)]^2 (118)}{(38)(80)(67)(51)}$$

Degrees of Freedom = 1

$\chi^2 = 11.6339$

alpha between .0010 -- .0005

than one disease entity. Inheritance as an autosomal recessive trait seems well established. However, two different autosomal recessive diseases with similar clinical findings would be distinguishable only by 1) biochemical methods, or 2) a mating of two affected individuals resulting in normal offspring.

B. α_1-ANTITRYPSIN

At the alpha-1-antitrypsin locus, the ZZ genotype is associated with emphysema, a pulmonary disorder.[66] Only two individuals in this study have decreased amounts of alpha-1-antitrypsin, a father and a paternal grandmother, in two different families; they probably have the MZ genotype. Thus the alpha-1-antitrypsin deficiency alleles appear to have no association with the occurrence of cystic fibrosis.

C. LINKAGE RESULTS BETWEEN CYSTIC FIBROSIS AND THE TEST LOCI

Another approach to improve diagnosis and treatment of cystic fibrosis individuals is to identify a linkage of the cystic fibrosis locus with another locus. Typing of the HLA loci is already used for diagnosis of a number of other inherited disorders. Several components of the complement system, including C2, C4 and C8 are known to be closely linked to the HLA loci.[67, 68] Juvenile diabetes mellitus[69, 70], hemochromatosis[71] and 21-hydroxylase deficiency[53] are also known to be closely linked to the HLA system.

The highest total lod score found in this study was +2.0272, for 0.00 map units distance, between the cystic fibrosis and Kell loci. This lod score represents a probability of 118 to 1 that linkage is present between the cystic fibrosis and Kell loci. The accepted "cut-off" value for linkage is +3; this represents odds of 1000 to 1 that the conclusion of linkage is correct. At odds of 118 to 1, the 1 represents .85% chance that linkage is not present between the two loci, but only appears to be present due to some unknown bias or error in population selection or experimental design.

Data for linkage of the cystic fibrosis and Kell loci is also of interest because absolute linkage cannot be excluded by this study. Absolute linkage would indicate that the cystic fibrosis and Kell loci occupy the same location of the human genome, or that they occupy different locations but are controlled by a single expressor region. However, absolute linkage may be eliminated by the study of Edwards (Table 7). Scores for 17 families scored for segregation of the father gave total lod scores of -1.154 and -0.225 at map distances of 0.10 and 0.30 Morgans, respectively. At 0.00 Morgans map distance, the score of these 17 families would approach negative infinity. (Figure 12).

A known linkage of the cystic fibrosis locus to a test locus such as Kell has a variety of practical uses. 1) In families where a cystic fibrosis individual has previously occurred, at-risk infants could be screened at birth for the test locus, and therapy initiated immediately for affected individuals. 2) Siblings of patients could be screened for carrier status; Mendelian risks of sibling carriers

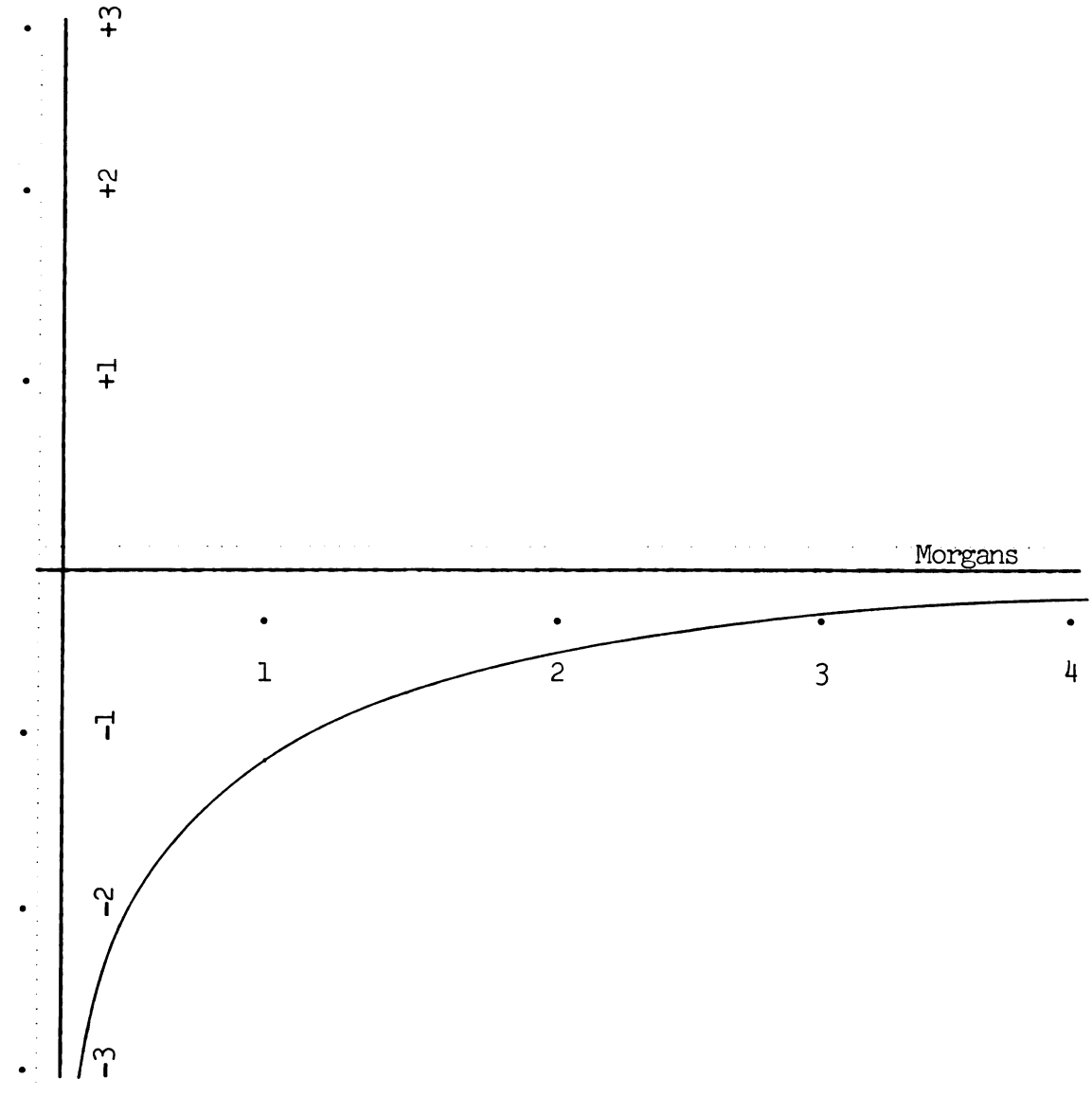

FIGURE 12. LOD SCORE TOTAL FOR 17 FAMILIES SCORED FOR SEGREGATION OF THE FATHERS ONLY, BY EDWARDS[115]

are 67%; risks based on determination of a linked test locus would approach 0% or 100% for many siblings. 3) At risk parents would have the option of in utero testing and subsequent abortion of high risk fetuses for a test locus that could be determined by amniocentesis. 4) A known linkage of cystic fibrosis would permit further linkage studies, first to localize the cystic fibrosis locus on the human genome and second with the cystic fibrosis locus as a relatively common test locus.

Limitations of the use of a test locus for diagnosis of cystic fibrosis and identification of carriers are 1) the alleles of the test locus must visibly segregate in the family (i.e. at the Kell locus, a kk x kk mating gives no diagnostic information), 2) the test locus must be close to the cystic fibrosis locus; the greater the distance separating the two loci, the greater the probability of a recombinant event occurring between them, and 3) a highly polymorphic locus, such as the HLA locus will yield more information than a test locus such as Kell with only two common alleles; for example at the HLA loci, it can be determined exactly which alleles of each child came from each parent in almost all families.

A number of areas of the human linkage map may be excluded from linkage with the cystic fibrosis locus by this study. (I.e., when the lod score for all families is less than -2, linkage is excluded with a probability of 100 to 1.) The cystic fibrosis locus is excluded from a distance of .14 map units or less on either side of the histocompatibility complex,..09 map units or less from the ABO locus

and the Rhesus loci, .05 map units or less from the MNSs loci, .04 map units or less from the P locus and .01 map units or less from the Lewis, Secretor, Kidd and Duffy loci. In all a total of .90 map units are shown not to contain the cystic fibrosis locus.

Absolute linkage (linkage at 0.00 map units distance) is excluded for each of the above loci. However, any of these loci may be linked to the cystic fibrosis locus at a greater map distance. For example, although linkage of the cystic fibrosis and HLA loci was excluded from 0.00 to 0.14 map units, they may be linked at .30 map units. Linkage could be either excluded or confirmed at .30 map units by scoring additional families until a total lod score of either -2 or +3, respectively, was reached.

These chromosomal regions where linkage can neither be confirmed nor denied are "grey" areas, where the cystic fibrosis locus may or may not be located. All other areas of the human autosome not tested by this study also remain as "grey" areas until data is gathered to either confirm or deny linkage with the cystic fibrosis locus.

D. COMBINED LOD SCORES OF THIS STUDY WITH SCORES OF OTHER PUBLISHED STUDIES

Addition of lod scores of this study with lod scores of other published studies increases the information available.[29, 30, 48] (Tables 7 and 24, Figures 13 - 14). Combining lod scores of all families for this study and the other studies does not result in a confirmed linkage of the

TABLE 24. TOTALS OF LOD SCORES OF THE BLOOD GROUP LOCI FROM THIS STUDY AND FROM OTHER PUBLISHED STUDIES. (SEE TABLE 7.)

		Map Distances, Centimorgans					
		0.00	0.05	0.10	0.20	0.30	0.40
HLA	Male			-2.521		.378	
ABO	Male			-4.329		- .423	
MNS	Female	-∞	- .6777	.1410	.5503	.3444	
	Male	-∞	-1.9560	- .7747	.0052	.1198	
	Both	-∞	-1.9347	.0994	1.1763	1.0380	
	Total	-∞	-4.5166	- .9505	.9214	.9195	
	Female		- .1644	.9123		.7747	
	Male		-3.3824	-1.2877		.2361	
	Both		-2.8478	.3577		1.5846	
	Total		-7.1472	-1.2246		1.4474	
	Total			-1.071		1.781	
Kell	Male			.387		.306	
Kidd	Male			- .467		- .065	
Duffy	Male			- .671		.232	

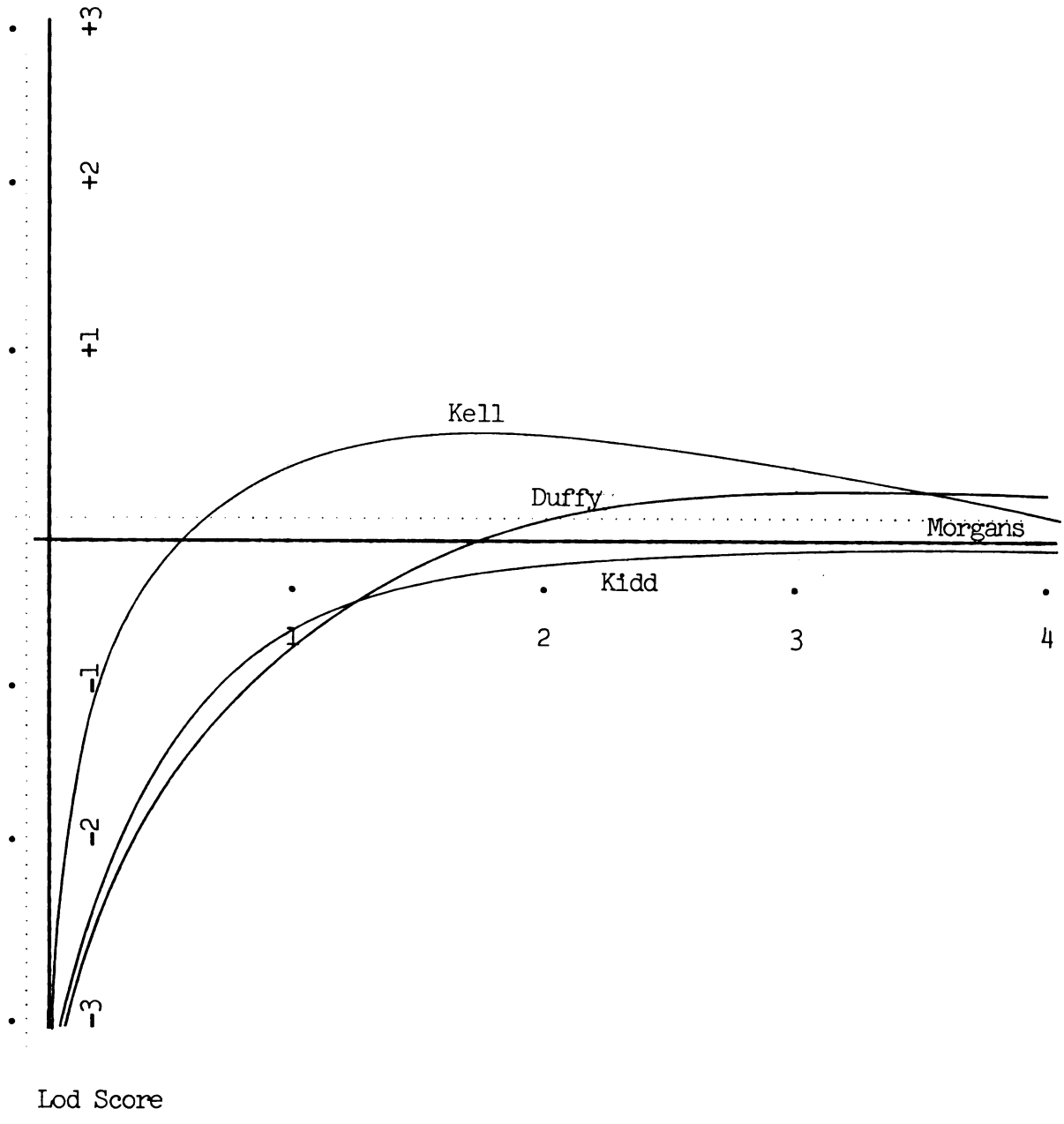

FIGURE 13. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE DUFFY, KELL AND KIDD BLOOD GROUPS--COMBINED RESULTS OF THIS STUDY WITH OTHER PUBLISHED STUDIES.

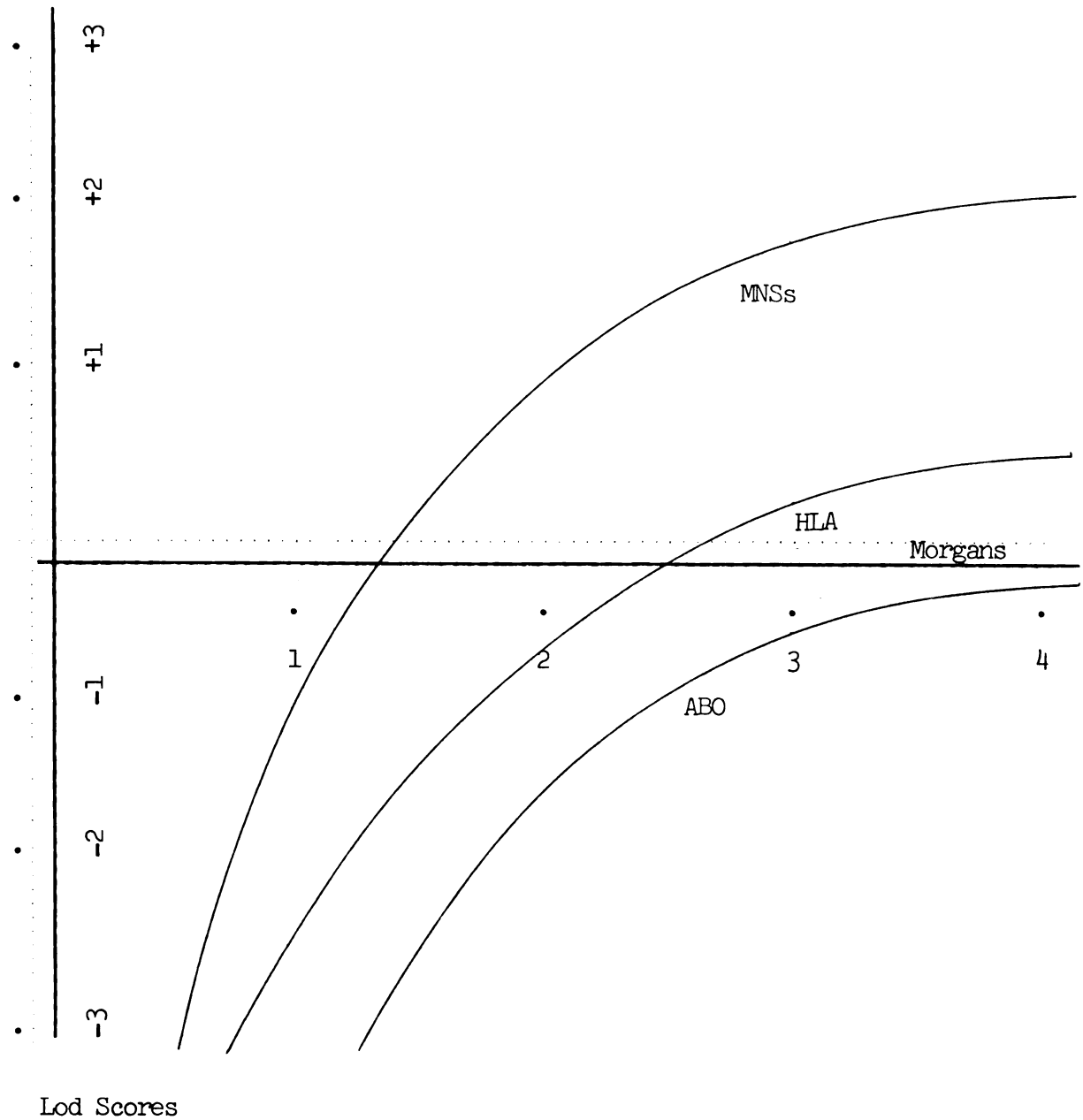

FIGURE 14. LOD SCORE TOTALS BETWEEN CYSTIC FIBROSIS AND THE MNSs, HLA AND ABO BLOOD GROUPS--COMBINED RESULTS OF THIS STUDY WITH OTHER PUBLISHED STUDIES.

cystic fibrosis locus. However, the cystic fibrosis locus can be excluded from greater areas of the human gene map; from .20 map units or less on either side of the ABO locus, .04 map units or less from the Duffy locus and .08 map units or less from the MNSs locus. These combined studies exclude the cystic fibrosis locus from 1.22 map units of the total human genome.

This study indicates a possible linkage of the cystic fibrosis and Kell loci. After combining lod scores of this study and the study of Edwards,[48] at the Kell locus, the total lod score at 0.10 map units was 0.387, and at 0.30 map units was 0.306. (Graphically the scores of Edwards indicate exclusion of absolute linkage between Kell and cystic fibrosis.) (Figure 13).

The possibility of more than one cystic fibrosis locus (or allele) has still not been excluded. If "cystic fibrosis" is a composite of more than one disease process, then any linkage studies would have to be modified to address linkage of several cystic fibrosis loci or of a multiallelic cystic fibrosis locus. While current data still support autosomal recessive inheritance, conclusive proof depends on either a confirmed mating of two cystic fibrosis individuals or identification of the basic metabolic defect responsible for the clinical symptoms of cystic fibrosis.

V. CONCLUSION

A total of fifty-nine families were studied for linkage and for association of the cystic fibrosis locus with 15 red and white blood cell loci. Linkage was not confirmed between cystic fibrosis and any of the test loci. Areas of the human linkage map that may be excluded from linkage with the cystic fibrosis locus by this study are: .14 map units or less from the histocompatibility loci, .09 map units or less from the ABO locus and the Rhesus loci, .05 map units or less from the MNSs loci, .04 map units or less from the P locus and .01 map units or less from the Lewis, Secretor, Kidd and Duffy loci. In all, a total of .90 map units are shown not to contain the cystic fibrosis locus. A significant association was found between the cystic fibrosis locus and the HLA-B7 antigen. (α between .0010 and .0005). No association was found between cystic fibrosis and deficiency of alpha-1-antitrypsin.

APPENDICES

APPENDIX A

Family and individual identification numbers and intrafamilial relationships.

Family Number	Individual Number	Relation to Affected Child (*)
B01	B011	* affected female
	B012	mother
	B013	father
	B014	brother
	B015	brother
B02	B021	father
	B022	mother
	B023	* affected female
	B024	brother
	B025	brother
	B026	brother
	B027	brother
	B028	brother
B03	B031	* affected female
	B032	mother
	B033	sister
B04	B041	* affected female
	B042	mother
B05	B051	mother
	B052	* affected female
	B053	maternal grandmother
	B054	half-brother (same mother as *)
	B055	maternal grandfather
A06	A061	* affected male
	A062	father
	A063	sister
	A064	sister
	A065	sister
	A066	brother
	A067	mother
B07	B071	* affected male
	B072	mother
	B073	father
	B074	maternal grandmother
B08	B081	father
	B082	* affected female

B09	B091	* affected male
	B092	mother
A10	A101	father
	A102	* affected female
A11	A111	* affected female
	A112	mother
A12	A121	mother
	A122	* affected male
A13	A131	* affected male
	A132	mother
	A133	father
A14	A141	* affected male
	A142	brother
	A143	father
	A144	sister
	A145	sister
	A146	brother
A15	A151	mother
	A152	* affected female
	A153	* affected female
	A154	father
	A155	paternal grandmother
	A156	paternal grandfather
	A157	brother
	A158	maternal grandfather
	A159	maternal grandmother
A16	A161	mother
	A162	paternal grandmother
	A163	* affected male
	A164	father
	A165	sister
B17	B171	mother
	B172	* affected male
	B173	father
B18	B181	* affected female
	B182	mother
B19	B191	* affected female
	B192	mother
	B193	* affected male
	B194	sister
	B195	father
	B196	brother
	B197	paternal grandmother
	B198	maternal grandmother

B20	B201	mother
	B202	* affected male
B21	B211	* affected male
	B212	mother
	B213	sister
	B214	father
B22	B221	sister
	B222	* affected male
B23	B231	* affected female
	B232	mother
	B233	brother
	B234	* affected male
B24	B241	* affected female
	B242	* affected male
	B243	mother
	B244	brother
	B245	brother
	B246	brother
	B247	sister
	B248	sister
	B249	maternal grandmother
	B241-1	brother
	B242-1	father
	B243-1	brother
A25	A251	mother
	A252	* affected female
A26	A261	* affected female
	A262	mother
	A263	maternal grandfather
	A264	maternal grandmother
	A265	father
	A266	brother
	A267	brother
B27	B271	mother
	B272	* affected male
	B273	* affected male
	B274	father
	B275	sister
B28	B281	mother
	B282	* affected male
B29	B291	* affected female
	B292	mother
	B293	father
	B294	maternal grandfather
	B295	maternal grandmother

B30	B301	* affected male
	B302	mother
	B303	brother
B31	B311	* affected male
	B312	father
	B313	mother
	B314	brother
	B315	maternal grandmother
	B316	maternal grandfather
B32	B321	* affected male
	B322	mother
	B323	father
	B324	brother
	B325	half-sister (same mother as *)
B33	B331	* affected male
	B332	maternal grandmother
	B333	mother
	B334	father
	B335	maternal grandfather
A34	A341	* affected female
	A342	mother
	A343	maternal grandmother
	A344	paternal grandmother
	A345	father
	A347	maternal grandmother's mother
	A348	maternal grandfather
A35	A351	* affected male
	A352	father
	A353	mother
B36	B361	father
	B362	sister
	B363	* affected male
	B364	* affected male
	B365	mother
	B366	sister
	B368	maternal grandfather
	B369	maternal grandmother
B37	B371	mother
	B372	* affected female
	B373	brother
	B374	father
	B375	paternal grandmother

B38	B381	brother
	B382	sister
	B383	sister
	B384	mother
	B385	father
	B386	* affected male
	B387	maternal grandmother
B39	B391	mother
	B392	brother
	B393	father
	B394	brother
	B395	sister
	B396	brother
	B397	* affected male
B40	B401	mother
	B402	* affected male
B41	B411	* affected female
	B412	brother
	B413	mother
B42	B421	* affected female
	B422	* affected male
	B423	father
	B424	mother
	B425	sister
A43	A431	mother
	A432	* affected male
B44	B441	* affected female
	B442	father
	B443	paternal grandmother
B45	B451	* affected female
	B452	mother
	B453	father
	B454	maternal grandfather
	B455	maternal grandmother
	B456	paternal grandmother
	B457	paternal grandfather
B46	B461	* affected male
	B462	mother
	B463	father

A47	A471	mother
	A472	father
	A473	brother
	A474	* affected female
	A475	* affected male
A48	A481	* affected female
	A482	mother
	A483	sister
	A484	father
A49	A491	brother
	A492	* affected male
	A493	mother
A50	A501	mother
	A502	father
	A503	* affected female
C51	C511	* affected female
	C512	father
C52	C521	mother
	C522	* affected female
C53	C531	* affected male
	C532	father
C54	C541	* affected male
	C542	father
C55	C551	mother
	C552	* affected female
	C553	maternal grandmother
C56	C561	* affected female
	C562	mother
C57	C571	* affected female
	C572	father
C58	C581	father
	C582	mother
	C583	* affected male
C59	C591	* affected female
	C592	father
	C593	mother
	C594	half-sister (mother same as *)
	C595	half-sister (mother same as *)

APPENDIX B

HLA haplotypes of each individual. (* affected child).

Individual Number	First Haplotype	Second Haplotype	
B011*	A3/B40	A32/B27	
B012	A32/B12	A32/B27	
B013	A1/B15	A3/B40	
B014	A3/B40	A32/B27	
B015	A3/B40	A32/B12	
B021	A1/B8	A2/B14	
B022	A2/B12	A24/B7	
B023*	A2/B12	A2/B14	
B024	A2/B14	A24/B7	
B025	A2/B12	A2/B14	
B026	A1/B8	A24/B7	
B027	A1/B8	A2/B12	
B028	A1/B8	A2/B12	
B031*	A24/B27	A32/B14	
B032	A2/B7	A32/B14	
B033	A2/B7	A24/B27	
B041*	A1/B7	A2/B15	
B042	A2/B15	A3/B35	C4
B051	A2/B39	A23/B14	
B052*	A2/B39	A25/B40	
B053	A2/B37	A23/B14	
B054	A-/B12	A2/B39	
B055	A2/B39	A26/B7	
A061*	A2/B12	A33/B14	
A062	A3/B7	A33/B14	
A063	A2/B27/C2	A33/B14	
A064	A2/B27/C2	A3/B7	
A065	A2/B27/C2	A3/B7	
A066	A2/B27/C2	A33/B14	
A067	A2/B12	A2/B27/C2	
B071*	A1/B7	A2/B5	
B072	A1/B7	A3/B15	
B073	A2/B5	A11/B39	C2
B074	A3/B15	A24/B12	C2, C3
B081	A1/B8	A2/B15	
B082*	A-/B35	A2/B15	C4
B091*	A1, A2, B15, B22		
B092	A1, A2, B-, B22		

A101	A-/B-	A29/B17	
A102*	A2/B15	A29/B17	
A111*	A1/B8	A28/B35	
A112	A2/B16	A28/B35	
A121	A-/B8	A2/B15	
A122*	A2/B15	A3/B40	
A131*	A10/B7	A32/B12	
A132	A10/B7	A11/B12	
A133	A11/B27	A32/B12	
A141*	A2/B15	A24/B21	
A142	A2/B15	A2/B21	
A143	A2/B21	A24/B21	
A144	A2/B15/C3	A24/B21	
A145	A2/B15/C3	A24/B21	
B146	A2/B35/C4	A24/B21	
A151	A1/B17	A28/B35	
A152*	A1/B17	A23/B12	
A153*	A23/B12	A28/B35	
A154	A2/B7	A23/B12	
A155	A23/B12	A28/B35	
A156	A2/B7	A28/B40	
A157	A1/B17	A23/B12	C4
A158	A3/B18	A28/B35	C4
A159	A1/B17	A3/B35	
A161	A28, A30, B12, B35		
A162	A2/B35	A19/B40	
A163*	A2/B35	A30/B12	
A164	A2/B7	A2/B35	
A165	A2/B7	A30/B35	
B171	A2/B5	A24/B40	
B172*	A2/B5	A2/B21	
B173	A2/B21	A3/B17	
B181*	A2/B13	A3/B7	
B182	A3/B7	A28/B-	
B191*	A2/B40	A28/B5	
B192	A2/B12	A28/B5	
B193*	A2/B12	A2/B40	
B194	A2/B40	A28/B5	C1
B195	A2/B12	A2/B40	
B196	A2/B12	A28/B5	
B197	A-/B7	A2/B12	
B198	A2/B12	A3/B18	

B201	A2/B27	A24/B35	
B202*	A1/B8	A24/B35	
B211*	A2/B7	A2/B12	
B212	A2/B7	A24/B15	
B213	A11/B27	A24/B15	
B214	A2/B12	A11/B27	
B221	A2/B15	A10/B8	
B222*	A10/B8	A24/B35	
B231*	A2/B15	A30/B13	
B232	A11/B8	A30/B13	
B233	A2/B8	A30/B13	
B234*	A2/B15	A30/B13	
B241*	A2/B7	A29/B5	
B242*	A1/B8	A2/B7	
B243	A2/B7	A3/B7	
B244	A2/B7	A29/B5	
B245	A1/B8	A2/B7	
B246	A1/B8	A3/B7	
B247	A1/B8	A3/B7	
B248	A1/B8	A3/B7	
B249	A2/B7	A-/B15	
B241-1	A2/B7	A29/B5	
B242-1	A1/B8	A29/B5	
B243-1	A3/B7	A29/B5	
A251	A2/B15	A28/B13/C4	
A252*	A2/B15	A-/B18	
A261*	A2/B12	A3/B7	
A262	A2/B12	A-/B15	
A263	A-/B12	A-/B15/C3	
A264	A2/B12	A28/B15/C3	
A265	A2/B35/C4	A3/B7	
A266	A2/B12	A3/B7	
A267	A2/B12	A3/B7	
B271	A1/B7	A23/B8	
B272*	A2/B12	A23/B8	
B273*	A23/B8	A28/B39	
B274	A2/B13	A28/B39	
B275	A1/B7	A28/B39	
B281	A2/B13	A11/B7	
B282*	A-/B27	A2/B13	C1

B291*	A2/B12	A2/B12
B292	A1/B8	A2/B12
B293	A-/B40	A2/B12
B294	A2/B12	A3/B7
B295	A1/B8	A2/B13
B301*	A29, A30, B14, B40	
B302	A29, A32, B7, B40	
B303	A28, A29, B7, B12, B40	
B311*	A2/B12	A3/B15/C4
B312	A3/B35/C4	A29/B12
B313	A2/B12	A24/B21
B314	A3/B35	A24/B21
B315	A2/B14	A24/B21
B316	A1/B17	A2/B12
B321*	A1/B7	A3/B7
B322	A1/B7	A2/B5
B323	A3/B7	A24/B7
B324	A2/B5	A24/B7
B325	A2/B5	A26/B27
B331*	A29/B12	A32/B40
B332	A1/B8	A2/B12
B333	A1/B8	A32/B40
B334	A2/B40	A29/B12
B335	A29/B27	A32/B40
A341*	A2/B22	A32/B40
A342	A2/B22/C3	A11/B27
A343	A2/B22/C3	A2/B27/C2
A344	A2/A32/B15/C3	A25/B39
A345	A25/B39	A32/B40
A347	A2/B27/C2	A32/B35/C4
A348	A11/B27	A24/B8
A351*	A1/B37	A31/B21
A352	A1/B37	A2/B38
A353	A28/B12	A31/B21
B361	A2/B5	A29/B12
B362	A2/B12	A29/B12
B363*	A2/B12	A29/B12
B364*	A2/B12	A29/B12
B365	A2/B12	A2/B21
B366	A2/B12	A29/B12
B368	A2/B21	A24/B12/C1
B369	A2/B12	A3/B7

B371	A3/B12	A32/B35/C4	
B372*	A2/B22/C3	A3/B12	
B373	A3/B7	A3/B12	
B374	A2/B22/C3	A3/B7	
B375	A-/B35/C4	A3/B7	
B381	A2/B7	A26/B22	
B382	A2/B7	A26/B22	
B383	A2/B7	A26/B22	
B384	A2/B37	A26/B22	
B385	A2/B7	A3/B39	
B386*	A2/B37	A3/B39	
B387	A26/B22	A29/B18	
B391	A11/B7	A32/B40	
B392	A1/B35	A32/B40	
B393	A1/B35	A24/B7	
B394	A1/B35	A32/B40	
B395	A24/B7	A32/B40	
B396	A11/B7	A24/B7	
B397*	A1/B35	A11/B7	
B401	A29/B12	A32/B15	
B402*	A3/B40	A29/B12	
B411*	A3/B12	A33/B7	
B412	A2/B18	A28/B13	
B413	A2/B18	A3/B12	
B421*	A28/B35	A29/B12	
B422*	A2/B12	A29/B12	
B423	A24/B7	A29/B12	
B424	A2/B12	A28/B35	C4
B425	A2/B12	A29/B12	
A431	A2, A-, B12, B15, C2, C3		
A432*	A2, A9, B12, B15, C3, C4		
B441*	A-/B18	A1/B8	
B442	A1/B8	A26/B27/C2	
B443	A24/B15	A26/B27	
B451*	A29/B12	A31/B35/C4	
B452	A25/B17	A29/B12	
B453	A10/B8	A31/B35/C4	
B454	A25/B17	A32/B40	
B455	A1/B-	A29/B12	
B456	A2/B12	A10/B8	
B457	A1/B17	A31/B35/C4	
B461*	A24/B37	A24/B40	
B462	A2/B12	A24/B37	
B463	A24/B40	A26/B39	

A471	A2/B-	A25A32/B17
A472	A1/B38	A2/B12
A473	A1/B38	A2/B-
A474*	A2/B12	A25A32/B17
A475*	A1/B38	A2/B-
A481*	A1/B8	A2/B5
A482	A2/B5	A-/B-
A483	A1/B8	A2/B5
A484	A1/B8	A3/B22/C3
A491	A2, A3, B7, B15, C4	
A492*	A2, A29, B12, B15	
A493	A2, A3, B7, B-	
A501	A2/B12	A29/B15
A502	A1/B17	A2/B12
A503*	A1/B17	A29/B15
C511*	A2/B5	A25/B18
C512	A1/B12	A25/B18
C521	A2/B12	A3/B5
C522*	A-/B14	A3/B5
C531*	A-/B-	A24/B40/C3
C532	A2/B12	A24/B40/C3
C541*	A2/B12	A32/B35/C4
C542	A2/B12	A24/B21/C1
C551	A2/B12	A3/B7
C552*	A-/B-	A2/B12
C553	A2/B12	A24/B7
C561*	A1/B37	A28/B12
C562	A1/B37	A2/B27/C1
C571*	A2/B12	A3/B7
C572	A-/B40	A2/B12
C581	A1/B13	A11/B7
C582	Insufficient sample to type	
C583*	A3/B-	A11/B7
C591*	A26/B27/C1	A29/B12
C592	A2/B18	A26/B27/C1
C593	A2/B15/C3	A29/B12
C594	A2/B15/C3	A24/B35/C4
C595	A24/B35	A29/B12

APPENDIX C

Results of red cell analysis.

	A	B	A_1	D	C	E	c	e	D^u	M	N	S	s	Le^aLe^b	K	k	P_1	Fy^aFy^b	Jk^a
*B011	+	+	+	+	+	+	+	+	N	+	+	+	+	- -	-	N	+	- N	+
B012	+	-	+	+	-	+	+	+	N	+	+	+	+	- -	-	N	+	- N	+
B013	+	+	N	+	+	-	-	+	N	+	+	-	+	- +	-	N	+	- N	+
B014	+	+	N	+	+	+	+	+	N	+	-	+	-	- +	-	N	-	- N	+
B015	+	-	+	+	+	-	+	+	N	+	+	-	+	- +	-	N	-	- N	+
B021	-	-	N	-	-	-	+	+	-	+	-	+	-	+ -	-	N	+	- N	+
B022	+	-	+	-	-	-	+	+	-	+	+	+	+	- +	-	N	-	+ N	+
*B023	+	-	+	-	-	-	+	+	-	+	-	+	-	- +	-	N	+	- N	-
B024	-	-	N	-	-	-	+	N	-	+	+	+	+	- -	-	N	+	- N	+
B025	-	-	N	-	-	-	+	N	-	+	-	+	-	- +	-	N	+	- N	+
B026	+	-	+	-	-	-	+	N	-	+	-	+	-	- -	-	N	+	- N	+
B027	+	-	+	-	-	-	+	N	-	+	+	+	+	- +	-	N	+	- N	+
B028	-	-	N	-	-	-	+	N	-	+	-	+	-	- -	-	N	-	+ N	+
*B031	+	-	-	+	+	-	+	+	N	+	-	-	+	- +	-	N	+	- N	+
B032	-	+	N	+	-	+	+	+	N	+	-	-	+	- +	-	N	+	- N	+
B033	-	+	N	+	+	-	+	N	N	+	-	-	+	+ -	-	N	+	- N	+
*B041	-	-	N	+	-	+	+	+	N	+	-	+	-	- +	-	N	+	- N	+
B042	-	-	N	+	-	+	+	+	N	+	-	+	+	- +	-	N	+	+ N	+
B051	+	-	+	+	+	-	-	+	N	+	+	-	+	- +	-	N	+	+ N	-
*B052	-	-	N	+	+	-	-	+	N	+	-	-	+	- +	-	N	+	+ N	+
B053	+	-	+	+	+	-	-	+	N	+	-	+	-	- +	-	N	+	+ -	-
B054	+	-	+	-	+	-	+	+	-	+	+	-	+	- +	-	N	+	- +	-
B055	+	-	-	-	+	-	+	+	-	-	+	-	+	- +	-	N	+	+ +	-
*A061	-	-	N	+	+	-	+	+	N	+	-	+	+	+ -	-	N	+	+ +	+
A062	-	-	N	+	-	-	+	+	N	+	-	-	+	- -	+	+	+	- +	+
A063	-	-	N	+	-	-	+	N	N	+	-	+	+	+ -	+	+	+	+ N	+
A064	-	-	N	+	-	-	+	N	N	+	-	+	+	- +	-	N	+	- N	+
A065	-	-	N	+	-	-	+	N	N	+	-	+	+	- +	+	+	+	- N	-
A066	-	-	N	+	+	-	+	N	N	+	-	+	+	+ -	+	+	+	+ N	+
A067	-	-	N	+	+	-	+	N	N	+	-	+	-	- +	-	N	+	+ N	+
*B071	+	-	-	+	+	+	+	+	N	-	+	+	+	- -	-	N	+	+ +	+
B072	-	-	N	-	-	+	+	+	-	+	+	+	-	- +	-	N	+	- +	+
B073	+	-	-	+	+	-	+	+	N	+	+	+	-	+ -	-	N	+	+ +	+
B074	-	-	N	-	-	-	+	N	-	+	+	+	+	- +	-	N	-	+ N	+
B081	+	-	+	+	+	-	+	+	N	+	-	+	+	- -	+	+	+	- +	-
*B082	+	-	+	+	+	-	+	+	N	+	+	+	-	- +	+	+	+	- +	+
*B091	-	-	N	+	-	+	+	+	N	+	-	+	+	- +	-	N	+	- +	+
B092	-	-	N	+	-	+	+	+	N	+	-	+	+	- +	-	N	+	+ +	+

	A	B	A_1	D	C	E	c	e	D^u	M	N	S	s	Le^aLe^b	K	k	P_1	Fy^aFy^b	Jk^a
A101	-	-	N	+	+	-	+	+	N	+	-	+	+	- +	-	N	+	+ -	+
*A102	-	-	N	+	-	+	+	+	N	+	+	+	+	+ -	-	N	+	+ -	+
*A111	+	-	-	+	+	+	+	+	N	+	+	+	+	- +	+	+	+	+ +	+
A112	-	-	N	+	+	-	-	+	N	-	+	-	+	- +	-	N	+	+ -	+
A121	+	-	+	+	+	-	-	+	N	+	-	+	+	- +	-	N	+	+ +	+
*A122	-	-	N	+	+	-	-	+	N	+	-	-	+	- +	-	N	+	+ +	+
*A131	-	-	N	+	+	-	+	+	N	-	+	-	+	+ -	-	N	-	+ +	+
A132	-	-	N	-	-	-	+	+	-	-	+	-	+	- +	-	N	-	+ +	+
A133	+	-	+	+	+	-	-	+	N	+	+	-	+	+ -	-	N	+	- +	+
*A141	+	-	N	-	-	-	+	+	-	-	+	+	+	- +	-	N	+	+ +	-
A142	-	-	N	-	-	+	+	+	-	+	+	+	-	- +	-	N	+	- +	-
A143	+	-	N	-	-	+	+	+	-	+	+	+	-	+ -	-	N	+	- +	+
A144	+	-	+	-	-	-	+	N	-	+	-	+	-	- +	-	N	+	+ N	+
A145	+	-	+	-	-	-	+	N	-	+	+	+	-	- -	-	N	-	- N	+
A146	+	-	+	-	-	-	+	N	-	+	+	+	+	- +	-	N	+	- N	+
A151	+	-	N	+	+	-	-	+	N	+	+	+	+	- +	-	N	+	+ +	+
*A152	+	-	N	+	+	-	-	+	N	+	+	+	-	- +	-	N	+	- +	+
*A153	+	-	N	+	+	-	-	+	N	+	-	-	+	- +	-	N	-	- +	+
A154	+	-	+	+	+	-	-	+	N	+	-	+	+	- +	+	+	+	+ -	-
A155	+	-	+	+	+	-	-	+	N	+	+	-	+	- -	-	N	-	- N	+
A156	-	-	N	+	+	-	+	+	N	+	+	+	+	- +	+	+	+	+ N	+
A157	-	-	N	+	+	-	+	+	N	+	+	+	-	- +	-	N	+	+ N	+
A158	-	-	N	+	+	-	+	N	N	+	+	+	-	- +	-	N	+	+ N	+
A159	+	-	+	+	+	-	-	N	N	+	-	+	+	- +	-	N	-	+ N	+
A161	-	-	N	+	+	-	+	+	N	+	-	-	+	+ -	-	N	+	- +	+
A162	-	-	N	+	-	-	+	+	N	+	-	-	+	- +	-	N	+	- +	+
*A163	-	-	N	+	+	-	+	+	N	+	-	-	+	+ -	-	N	+	- +	+
A164	-	-	N	+	-	-	+	+	N	+	-	-	+	+ -	-	N	+	- +	+
A165	-	-	N	N	N	N	N	N	N	N	N	N	N	N N	N	N	N	N N	N
B171	+	-	+	+	+	-	+	+	N	+	+	-	+	- -	-	N	+	- +	+
*B172	+	-	+	-	-	-	+	+	-	+	-	+	+	- -	-	N	+	+ +	+
B173	+	-	+	-	-	-	+	+	-	+	+	+	+	- +	-	N	+	+ -	+
*B181	-	-	N	+	-	+	+	+	N	+	-	+	-	- -	-	N	+	+ +	+
B182	-	+	N	+	+	-	+	+	N	+	+	+	+	- +	-	N	+	+ +	+
*B191	+	-	-	+	-	-	+	+	N	+	-	+	+	- +	-	N	+	+ +	+
B192	-	-	N	-	-	-	+	+	-	+	-	+	+	- +	-	N	+	- +	-
*B193	+	-	-	+	+	-	+	+	N	+	-	+	-	- -	-	N	+	- +	+
B194	-	-	N	+	-	-	+	+	N	+	-	-	+	- +	-	N	+	+ +	+
B195	+	-	-	+	+	-	+	+	N	+	-	+	-	- -	-	N	+	+ N	+
B196	+	-	-	+	+	-	+	+	N	+	-	-	+	- +	-	N	+	- N	+
B197	+	-	-	+	+	-	-	N	N	+	-	+	+	- +	-	N	+	- N	+
B198	+	-	+	+	+	-	+	N	N	+	+	-	+	- +	-	N	+	+ N	+

	A	B	A_1	D	C	E	c	e	D^u	M	N	S	s	Le^a	Le^b	K	k	P_1	Fy^a	Fy^b	Jk^a
B201	+	-	-	+	+	-	-	+	N	+	+	-	+	-	+	-	N	+	+	+	+
*B202	+	-	+	+	+	-	-	+	N	-	+	-	+	-	+	+	+	+	+	-	-
*B211	+	-	-	+	+	-	+	+	N	+	+	-	+	-	+	-	N	+	-	+	+
B212	+	-	-	+	+	-	+	+	N	+	+	-	+	-	+	-	N	+	+	+	+
B213	-	-	N	+	+	-	+	N	N	-	+	-	+	-	+	-	N	+	+	N	+
B214	-	-	N	+	+	-	-	N	N	+	+	-	+	-	-	-	N	+	-	N	-
B221	-	+	N	+	+	-	-	+	N	+	-	+	+	-	+	-	N	+	-	+	+
*B222	-	-	N	+	+	-	+	+	N	+	+	+	+	-	+	-	N	+	-	+	-
*B231	-	-	N	+	-	+	+	-	N	+	-	+	+	+	-	-	N	+	+	+	-
B232	-	-	N	+	-	+	+	+	N	+	+	+	+	-	-	-	N	+	+	+	+
B233	+	-	+	+	-	+	+	+	N	+	+	-	+	-	-	-	N	+	+	+	+
*B234	+	-	+	+	-	+	+	+	N	+	+	+	+	+	-	-	N	+	-	+	-
*B241	-	-	N	+	+	-	-	+	N	+	+	+	+	-	+	+	+	+	-	+	+
*B242	-	-	N	+	+	-	-	+	N	+	+	+	+	-	+	+	+	+	-	+	+
B243	-	-	N	+	+	-	-	+	N	-	+	-	+	-	+	-	N	+	-	+	+
B244	-	-	N	+	+	-	-	+	N	+	+	+	+	-	+	-	N	+	-	N	+
B245	-	-	N	+	+	-	-	+	N	+	+	+	+	-	+	+	+	+	-	N	+
B246	-	-	N	+	+	-	-	+	N	+	+	+	+	-	+	-	N	+	-	N	+
B247	-	-	N	+	+	-	-	+	N	-	+	-	+	-	+	-	N	+	-	N	+
B248	-	-	N	+	+	-	-	+	N	+	+	+	+	-	+	-	N	+	-	N	+
B249	-	+	N	+	+	-	-	+	N	+	+	+	+	-	+	-	N	-	-	N	+
B241-1	-	-	N	+	+	-	-	+	N	+	+	+	+	-	+	-	N	+	-	N	+
B242-1	-	-	N	+	+	-	-	+	N	+	+	+	+	+	-	+	+	+	-	N	+
B243-1	-	-	N	+	+	-	-	+	N	+	+	+	+	-	+	-	N	+	-	N	+
A251	-	-	N	+	+	-	+	+	N	+	-	+	+	-	+	-	N	-	-	+	+
*A252	-	-	N	+	+	+	+	+	N	+	+	-	+	-	+	-	N	+	+	+	+
*A261	+	-	+	+	+	-	-	+	N	+	+	-	+	-	+	-	N	-	+	+	+
A262	-	+	N	+	+	-	-	+	N	+	+	-	+	-	+	-	N	+	-	+	+
A263	-	-	N	+	+	-	+	N	N	+	+	-	+	-	+	-	N	-	-	N	+
A264	-	+	N	+	+	-	-	N	N	+	+	-	+	-	+	-	N	+	-	N	+
A265	+	-	+	+	+	-	-	N	N	+	+	-	+	-	+	-	N	+	+	N	+
A266	-	+	N	+	+	-	-	N	N	+	-	-	+	-	+	-	N	+	+	N	+
A267	-	-	N	+	+	-	-	N	N	+	+	-	+	-	+	-	N	+	-	N	+
B271	+	-	+	-	-	-	+	+	-	+	+	+	+	-	+	-	N	+	-	+	-
*B272	+	-	+	+	+	-	+	+	N	-	+	-	+	-	+	-	N	+	-	+	-
*B273	+	-	+	+	+	-	+	+	N	+	+	+	+	-	+	-	N	+	-	+	+
B274	+	-	+	+	+	-	+	+	N	-	+	-	+	-	+	-	N	+	-	N	+
B275	+	-	+	+	+	-	+	+	N	+	+	+	+	-	+	-	N	+	-	N	-
B281	-	+	N	+	+	-	-	+	N	-	+	-	+	-	+	-	N	+	+	+	+
*B282	-	+	N	+	+	+	+	+	N	-	+	-	+	-	+	-	N	+	-	+	+

	A	B	A_1	D	C	E	c	e	D^u	M	N	S	s	Le^aLe^b	K	k	P_1	Fy^aFy^b	Jk^a
*B291	+	-	+	+	+	-	-	+	N	+	+	-	+	- +	-	N	+	+ +	+
B292	+	-	+	+	+	-	-	+	N	+	+	-	+	- +	-	N	+	+ +	+
B293	-	-	N	+	+	-	-	+	N	-	+	-	+	+ -	-	N	+	+ N	+
B294	+	-	+	+	+	-	-	+	N	+	+	-	+	+ -	-	N	+	+ +	+
B295	-	-	N	+	+	+	+	+	N	+	+	+	+	- +	-	N	+	- +	-
*B301	+	-	+	+	+	-	-	+	N	-	+	-	+	+ -	-	N	+	+ +	+
B302	-	-	N	+	+	-	+	+	N	-	+	-	+	- +	+	+	+	+ +	+
B303	+	-	+	+	+	-	+	N	N	+	+	+	-	- -	+	+	+	+ N	+
*B311	-	-	N	+	+	-	+	+	N	+	+	-	+	- +	+	+	-	+ +	+
B312	+	-	+	+	-	+	+	+	N	+	+	-	+	- +	-	N	+	- +	+
B313	-	-	N	+	+	-	+	N	N	+	+	-	+	- -	+	+	-	+ N	+
B314	+	-	+	+	-	+	+	+	N	-	+	-	+	+ -	-	N	+	+ N	+
B315	-	-	N	+	+	+	+	+	N	+	-	-	+	- +	-	N	+	+ N	+
B316	+	-	-	+	+	-	+	N	N	-	+	-	+	- +	+	+	+	- N	+
*B321	+	-	+	+	+	+	+	+	N	+	-	-	+	+ -	-	N	+	- +	-
B322	+	-	+	+	+	-	+	+	N	+	-	-	+	- +	-	N	+	- +	-
B323	+	-	+	+	+	+	+	+	N	+	-	+	-	- +	-	N	-	- N	+
B324	+	-	+	+	-	+	+	+	N	+	-	+	-	- +	-	N	-	- N	-
B325	+	-	+	+	+	-	-	N	N	+	+	-	+	- +	-	N	+	+ N	+
*B331	-	-	N	+	+	-	-	+	N	-	+	-	+	- -	-	N	+	- +	+
B322	-	-	N	+	+	-	+	+	N	+	+	-	+	- -	-	N	+	+ +	+
B333	-	-	N	+	+	-	-	+	N	+	+	-	+	- -	-	N	+	- +	+
B334	+	-	-	+	+	-	-	+	N	-	+	-	+	- -	-	N	+	- N	+
B335	+	-	+	+	+	+	+	+	N	-	+	-	+	- +	-	N	+	- N	+
*A341	+	-	+	+	+	-	+	+	N	+	-	-	+	+ -	-	N	+	+ -	+
A342	+	-	+	+	+	-	+	+	N	+	-	-	+	+ -	-	N	+	+ +	-
A343	+	-	-	+	+	-	-	N	N	+	-	-	+	- +	-	N	+	+ +	+
A344	-	-	N	-	-	-	+	N	-	+	+	-	+	- -	-	N	-	+ +	+
A345	-	-	N	-	-	-	+	+	-	+	-	-	+	+ -	-	N	+	+ -	+
A347	+	-	-	+	+	-	-	+	N	+	-	-	+	- -	-	N	+	+ N	+
A348	+	-	+	-	-	-	+	N	-	+	+	-	+	- +	-	N	+	- +	-
*A351	+	-	+	+	+	+	+	+	N	+	-	-	+	+ -	-	N	+	+ -	+
A352	+	-	+	+	-	+	+	+	N	+	+	-	+	- +	-	N	+	+ -	+
A353	+	-	+	+	+	-	-	N	N	+	+	-	+	+ -	-	N	+	+ +	-
B361	+	-	+	+	-	+	+	+	N	+	+	-	+	- +	-	N	+	- +	+
B362	-	-	N	+	+	+	+	+	N	-	+	-	+	- +	-	N	+	- +	+
*B363	-	-	N	+	+	-	+	+	N	+	+	-	+	- +	-	N	+	- +	+
*B364	+	-	+	+	+	+	+	+	N	-	+	-	+	+ -	-	N	+	- +	+
B365	-	-	N	+	+	-	-	+	N	+	+	-	+	- +	-	N	+	- -	+
B366	+	-	+	+	+	-	+	N	N	+	+	-	+	+ -	-	N	-	- N	+
B368	-	-	N	+	+	-	+	N	N	+	+	+	-	+ -	+	+	+	+ N	+
B369	-	-	N	+	+	+	+	+	N	-	+	-	+	- +	-	N	-	+ N	+

	A	B	A_1	D	C	E	c	e	D^u	M	N	S	s	Le^aLe^b	K	k	P_1	Fy^aFy^b	Jk^a
B371	-	+	N	+	+	-	+	N	N	+	+	+	+	- +	-	N	-	+ +	+
*B372	-	+	N	-	-	-	+	N	-	+	+	-	+	- +	-	N	-	+ +	+
B373	+	-	-	+	+	-	+	N	N	-	+	-	+	- +	+	+	-	+ -	+
B374	+	-	-	-	-	-	+	N	-	+	+	-	+	- +	+	+	+	+ -	+
B375	-	-	N	+	-	+	+	+	N	+	-	-	+	- +	-	N	+	+ +	+
B381	+	-	-	+	-	+	+	+	N	+	+	-	+	- +	-	N	+	+ +	-
B382	+	-	-	-	-	-	+	+	-	+	-	-	+	- +	-	N	+	+ +	-
B383	+	-	-	+	+	-	+	+	N	+	-	-	+	- +	-	N	+	+ +	-
B384	+	-	+	+	+	-	+	+	N	+	+	-	+	- +	-	N	+	+ -	-
B385	-	-	N	+	-	+	+	+	N	+	-	-	+	- -	-	N	+	- N	-
*B386	+	-	-	-	-	-	+	+	-	+	+	-	+	+ -	-	N	+	+ N	-
B387	+	-	+	+	+	+	+	+	N	-	+	-	+	- +	-	N	+	+ N	-
B391	-	-	N	+	-	+	+	+	N	-	+	-	+	+ -	+	N	-	- +	-
B392	-	-	N	+	-	+	+	-	N	+	+	+	+	- +	-	N	+	+ +	-
B393	-	+	N	+	-	+	+	+	N	+	-	+	-	- +	-	N	+	+ +	+
B394	-	-	N	+	-	+	+	-	N	+	+	+	+	- +	-	N	+	- +	-
B395	-	+	N	+	-	+	+	+	N	+	+	+	+	- +	-	N	+	+ +	-
B396	-	-	N	+	-	+	+	-	N	+	+	+	+	- +	-	N	+	+ +	+
*B397	-	-	N	+	-	+	+	+	N	+	+	+	+	- +	+	N	+	+ +	-
B401	-	+	N	+	+	-	+	N	N	+	-	+	+	- +	-	N	+	+ +	+
*B402	-	+	N	+	+	+	+	+	N	+	+	+	-	- +	-	N	+	+ +	+
*B411	+	-	+	+	-	+	+	+	N	-	+	-	+	- -	-	N	-	+ +	+
B412	+	-	+	+	+	-	+	N	N	+	-	+	+	+ -	-	N	-	+ +	+
B413	+	-	+	+	+	+	+	+	N	+	+	+	+	+ -	-	N	+	- +	-
*B421	-	-	N	-	-	-	+	N	-	-	+	-	+	- +	-	N	+	+ +	+
*B422	-	-	N	+	-	+	+	+	N	-	+	-	+	- +	-	N	-	+ +	+
B423	-	-	N	-	-	-	+	N	-	+	+	-	+	+ -	-	N	-	+ -	+
B424	-	-	N	+	-	+	+	+	N	-	+	-	+	- +	-	N	+	- +	+
B425	-	-	N	+	-	+	+	+	N	-	+	-	+	- +	-	N	+	+ +	+
A431	+	-	+	+	-	+	+	+	N	+	+	+	+	- +	-	N	+	+ -	+
*A432	+	-	+	-	-	-	+	N	N	+	+	+	+	- +	-	N	+	+ N	+
*B441	-	-	N	+	-	+	+	-	N	+	+	+	-	- +	-	N	+	- +	+
B442	-	-	N	+	-	+	+	+	N	+	-	+	+	+ -	-	N	+	- +	+
B443	-	-	N	+	+	-	+	N	N	+	+	+	+	- +	-	N	+	- N	+
*B451	+	-	+	-	-	-	+	-	-	-	+	-	+	+ -	-	N	+	+ N	+
B452	+	-	+	+	+	-	+	-	N	-	+	-	+	- +	-	N	+	+ N	+
B453	+	-	+	+	+	-	+	N	N	-	+	-	+	+ -	-	N	+	- N	+
B454	-	-	N	-	-	-	+	N	-	-	+	+	-	+ -	-	N	+	+ N	+
B455	+	+	+	+	+	-	+	N	N	-	+	-	+	- +	-	N	+	- N	-
B456	+	-	+	+	+	-	+	N	N	+	+	+	+	+ -	-	N	+	+ N	+
B457	-	-	N	-	-	-	+	N	-	-	+	-	+	- +	-	N	+	+ N	+

	A	B	A_1	D	C	E	c	e	D^u	M	N	S	s	Le^aLe^b	K	k	P_1	Fy^aFy^b	Jk^a
*B461	+	-	+	+	+	-	+	-	N	+	-	-	+	- +	-	N	+	+ N	+
B462	-	-	N	+	+	+	+	-	N	+	-	+	-	- +	-	N	+	+ N	+
B463	+	-	+	-	-	-	+	-	-	+	-	-	+	- +	-	N	+	+ N	+
A471	-	+	N	+	+	-	+	+	N	+	+	-	+	- +	-	N	+	+ N	+
A472	+	-	-	-	+	-	+	+	-	+	-	-	+	+ -	-	N	+	+ N	+
A473	-	-	N	-	+	-	+	+	-	+	-	-	+	- +	-	N	+	+ N	+
*A474	-	-	N	-	-	-	+	+	-	+	+	-	+	- +	-	N	+	+ N	+
*A475	+	+	-	+	+	-	+	+	N	+	+	-	+	- +	-	N	+	+ N	+
*A481	-	-	N	+	+	-	-	+	N	+	+	-	+	- +	-	N	-	+ N	-
A482	-	-	N	+	+	-	-	+	N	-	+	-	+	- +	-	N	-	+ N	+
A483	-	-	N	+	+	-	+	N	N	+	+	-	+	- +	-	N	+	+ N	-
A484	-	-	N	+	+	-	+	N	N	+	-	-	+	- -	-	N	+	+ N	-
A491	-	+	N	+	+	-	+	+	N	+	+	-	+	- +	-	N	-	+ N	+
*A492	-	-	N	+	+	-	+	+	N	+	-	-	+	- +	-	N	-	+ N	-
A493	-	+	N	+	+	-	-	+	N	+	+	-	-	- +	-	N	+	+ N	+
A501	+	-	+	+	+	-	-	+	N	+	+	-	-	- +	-	N	+	+ N	-
A502	+	-	-	+	+	-	+	+	N	+	-	-	+	- +	-	N	+	+ N	+
*A503	+	-	+	+	+	-	+	+	N	+	+	-	+	- +	-	N	+	+ N	+
*C511	-	-	N	+	+	-	-	N	N	+	-	+	-	- -	-	N	+	- N	+
C512	+	-	+	+	+	-	-	N	N	+	+	+	-	- +	-	N	+	- N	-
C521	-	-	N	-	-	-	+	N	-	+	+	+	+	- +	-	N	+	- N	+
*C522	-	-	N	+	+	-	+	N	N	-	+	-	+	+ -	-	N	-	+ N	+
*C531	-	-	N	+	+	-	-	N	N	+	+	-	+	- +	-	N	+	+ N	+
C532	-	-	N	+	+	-	+	N	N	+	+	+	+	- +	-	N	+	- N	+
*C541	+	-	+	-	-	-	+	N	-	+	+	-	+	- +	-	N	+	+ N	+
C542	+	-	+	+	+	-	+	N	N	+	-	-	+	- +	-	N	+	+ N	+
C551	-	-	N	+	+	-	+	N	N	-	+	-	+	- -	-	N	-	- N	-
*C552	-	-	N	-	-	-	+	N	-	-	+	-	+	- +	-	N	+	- N	+
C553	-	-	N	+	+	+	+	+	N	+	+	-	+	- +	-	N	-	- N	-
*C561	+	-	-	+	+	-	-	N	N	-	+	+	+	- +	-	N	+	- N	+
C562	-	-	N	+	+	-	+	N	N	+	+	+	-	- +	+	+	+	+ N	-
*C571	-	-	N	+	+	-	+	N	N	-	+	-	+	- +	-	N	+	+ N	+
C572	-	-	N	+	+	+	+	+	N	+	+	+	+	- -	-	N	-	- N	+
C581	-	+	N	+	+	-	+	N	N	+	+	+	-	- +	+	+	-	+ N	-
C582	-	-	N	+	+	-	+	N	N	+	-	+	+	+ -	-	N	-	+ N	+
*C583	-	+	N	+	+	-	+	N	N	+	+	+	+	- +	-	N	-	+ N	+

	A	B	A_1	D	C	E	c	e	D^u	M	N	S	s	Le^aLe^b	K	k	P_1	Fy^aFy^b	Jk^a
*C591	+	-	-	+	+	-	+	N	N	+	-	+	+	- +	-	N	+	+ N	+
C592	-	-	N	+	-	+	+	-	N	+	+	-	+	- +	-	N	+	+ N	+
C593	+	-	+	+	+	-	+	N	N	+	-	+	+	- +	-	N	+	- N	+
C594	+	-	-	+	+	-	-	N	N	+	-	+	-	- +	-	N	+	- N	-
C595	+	-	+	+	+	-	+	N	N	+	-	+	+	- +	-	N	+	- N	+

NOTE: * indicates an individual with cystic fibrosis.
+ indicates a positive result, i.e. the antigen is present.
- indicates a negative result, i.e. the antigen is not present.
N indicates the sample was not tested for that particular antigen.

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