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High Frequency of Nonclassical Steroid 21-Hydroxylase Deficiency

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SUMMARY

Nonclassical steroid 21-hydroxylase deficiency is an autosomal recessive disorder that is defined by clinical and hormonal criteria that distinguishes it from the classical 21-hydroxylase deficiency. No estimates of the gene frequency of nonclassical 21-hydroxylase deficiency, also called attenuated, late-onset, acquired, and cryptic adrenal hyperplasia, have been published thus far. Here, we have used *HLA-B* genotype data in families containing multiple members affected with nonclassical 21-hydroxylase deficiency together with the results of quantitative hormonal tests to arrive at estimates of gene and disease frequencies for this disorder. We found nonclassical 21-hydroxylase deficiency to be a far more common disorder than classical 21-hydroxylase deficiency, which occurs in 1/8,000 births. The prevalence of the disease in Ashkenazi Jews was 3.7%; in Hispanics, 1.9%; in Yugoslavs, 1.6%; in Italians, 0.3%; and in the diverse Caucasian population, 0.1%. The gene for nonclassical 21-hydroxylase deficiency is in genetic linkage disequilibrium with *HLA-B14* in Ashkenazi Jews, Hispanics, and Italians, but not in Yugoslavs or in a diverse, non-Jewish, Caucasian group. The penetrance of nonclassical 21-hydroxylase deficiency gene in the *HLA-B14* containing haplotypes was

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incomplete. Thus, nonclassical 21-hydroxylase deficiency is probably the most frequent autosomal recessive genetic disorder in man and is especially frequent in Ashkenazi Jews, Hispanics, Italians, and Yugoslavs.

INTRODUCTION

The classical form of congenital adrenal hyperplasia with steroid 21-hydroxylase deficiency is transmitted by an autosomal recessive gene [1] and is closely linked to the *HLA-B* locus on the short arm of chromosome 6 [2] with peak total lod scores as high as 9.5 [3] and 15.65 [4] for θ (recombinant fraction) = 0. Linkage disequilibrium with the *HLA-Bw47;DR7* haplotype segment has been demonstrated [5–7], and more recently the gene has been further localized to the C4 region of the *HLA* supergene by molecular genetic techniques [8, 9]. The frequency of the classical disease has been estimated at 1/8,000 by neonatal screening of a homogeneous Caucasian population in Italy using a microfilter paper technique [10]. In other studies throughout Europe and the United States, the frequency of the disease ranged from 1/5,000 to 1/15,000 [11]. A recent screening program for newborns in the state of Alaska using the microfilter paper technique revealed an extremely high incidence of 1/684 of classical 21-hydroxylase deficiency among Yupik-speaking Eskimos of western Alaska [12]. Thus, in Yupiks, this disorder is as common a human autosomal recessive genetic disorder as sickle cell anemia is in American blacks.

Nonclassical 21-hydroxylase deficiency, also called attenuated, late-onset, acquired, and cryptic adrenal hyperplasia, although suspected in the past as a cause of hirsutism in women [13], was first documented by hormonal measurements in 1957 [14]. Nonclassical 21-hydroxylase deficiency was demonstrated to be transmitted by an autosomal recessive gene and to be linked to *HLA* in 1980 [15]. Association with the *HLA-B14;DR1* haplotype segment has been noted, although linkage disequilibrium has not been rigorously established [15–18]. Because of differences in both hormonal response to ACTH stimulation and *HLA-B* associations, it has been suggested that the nonclassical disease gene may be an allelic variant of the classical form of the 21-hydroxylase deficiency gene [19]. In 1982, Kohn et al. presented detailed clinical and hormonal characterization of the symptomatic and asymptomatic forms of nonclassical 21-hydroxylase deficiency and calculated the peak total lod score for linkage to *HLA-B* as 3.575 at $\theta = 0$ [20].

To date, there have been no reports of the frequency of nonclassical 21-hydroxylase deficiency in the general population. Screening newborns using the microfilter paper method does not detect infants with nonclassical 21-hydroxylase deficiency, as serum 17-hydroxyprogesterone (17-OHP) concentrations are below the lower limit of the sensitivity of this assay [12]. We have therefore analyzed our patient population in an attempt to estimate the gene and disease frequency for nonclassical congenital adrenal hyperplasia due to

TABLE 1

21-HYDROXYLASE DEFICIENCY POPULATION

	Total no. patients	Total no. families	No. sib-pairs *HLA*-typed	No. *HLA-B* identical sib-pairs
Classical (all)	214	167*	30	30
Salt-wasting	116	89	12	12
Simple virilizing	94	76	16	16
Discordant†	4	2	2	2
Nonclassical (all)	100	43	18	14

* Seventeen of these families also have nonclassical patients.
† *HLA*-identical sibs, one with proven salt-wasting and the other with no evidence of salt-wasting.

steroid 21-hydroxylase deficiency. We have found a surprisingly high frequency of nonclassical 21-hydroxylase deficiency in the general population and especially in Ashkenazi Jews.

SUBJECTS, MATERIALS, AND METHODS

The population studied consisted of 214 patients from 167 families with the classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency and 100 patients from 43 families with the nonclassical disease (table 1). A total of 283 obligate heterozygote parents underwent ACTH stimulation tests. Eighty-seven percent of the classical families included in this study and 60% of the nonclassical families have been described [2–4, 7, 10, 15, 19–29]. Patients were of diverse ethnic backgrounds and were drawn from the United States, Europe, and Israel. The subjects were classified as nonclassical patients, classical patients, or heterozygotes, based on clinical and hormonal data. All patients underwent either 60-min ACTH, 0.25 mg Cortrosyn (Organon, West Orange, N.J.). IV bolus or 360-min ACTH, 0.40 mg Cortrosyn given continuously IV over 6 hrs. Radioimmunoassay of basal and stimulated serum 17-OHP, Δ4-androstenedione (Δ4), dehydroepiandrosterone (DHA), testosterone (T), desoxycorticosterone (DOC), corticosterone (B), cortisol (F), and aldosterone (aldo) was performed by methods described [30–32]. The coordinates of the basal and stimulated 17-OHP values were plotted on nomograms that permit assignment of subjects to the nonclassical, classical, heterozygote, or general population groups [28]. Patients with classical or nonclassical 21-hydroxylase deficiency and all heterozygous subjects are clearly distinguishable by these hormonal criteria; however, many members of the general population demonstrate ACTH-stimulated 17-OHP values in the range of obligate heterozygous subjects. Family members, other than parents of well-documented patients, in whom possible recombinations between the steroid 21-hydroxylase deficiency gene and the *HLA-B* or *-DR* locus could not be excluded were not used in the construction of the nomograms [28].

HLA typing was performed on peripheral blood lymphocytes for the antigens of the *A*, *B*, and *C* loci by the standard NIH two-stage microcytotoxicity test [33]. *DR* typing was performed by a modified complement-dependent cytotoxicity technique [34].

HLA gene frequencies were calculated by the counting method, that is, by determining the proportion of genes expressing a particular HLA-B antigen relative to the total number of antigens in a given patient group. *HLA-B* gene frequencies for the antigens B5, B8, B14, B35, B40, and Bw47, which have been previously demonstrated to deviate significantly in 21-hydroxylase deficiency syndromes from control groups, are shown in table 2*A* and *B*. Relative risk for the classical and nonclassical forms of 21-hydroxylase deficiency associated with a specific HLA antigen (table 3) was calculated by the

method of Woolf-Haldane [36]. Significance for the gene frequencies was calculated by the two-tailed Fisher exact method [37]. Conventional significance tests for relative risks were performed as summarized by Rubinstein [38]. The control population for each of the respective groups was ethnically matched. Because of the diverse composition of the group of "other Caucasians," there was no appropriate control group and statistical significance is not reported. Ninety-five percent confidence limits were calculated by the formula for a binomial distribution [39].

Gene frequency of the nonclassical disease was analyzed by two separate methods:

I. Hormonal Analysis

Since the disease is transmitted by an autosomal recessive gene, parents of a classical or nonclassical proband are obligate heterozygotes. The haplotypes of these obligate heterozygotes that are not transmitted to the respective probands represent an a priori random sample of the haplotypes in the population.

Families with offspring with classical 21-hydroxylase deficiency. If the proband has classical 21-hydroxylase deficiency, then the parents are obligate heterozygotes for the classical 21-hydroxylase deficiency, that is, they carry the classical 21-hydroxylase deficiency allele (fig. 1*A*). If one or both parents of a child with classical 21-hydroxylase deficiency manifest biochemical evidence of nonclassical 21-hydroxylase deficiency upon hormonal testing, then the allele not transmitted to the proband is, of necessity, a nonclassical allele (fig. 1*B*). Thus, these parents with nonclassical 21-hydroxylase deficiency represent compound heterozygotes, the genotype of which can be represented as 21-OH def^{severe}/21-OH def^{mild}. (Compound heterozygotes are thus defined as individuals who are heterozygous for two different abnormal alleles at the same locus.)

Families with offspring with nonclassical 21-hydroxylase deficiency. If the proband has nonclassical 21-hydroxylase deficiency, then the parents may be heterozygotes for

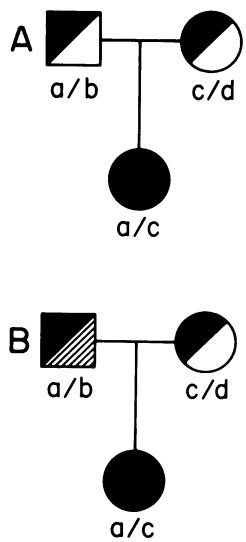

FIG. 1.—Possible 21-hydroxylase deficiency genotypes of parents with classical offspring. ▨ refers to nonclassical 21-hydroxylase deficiency haplotype (21-OH def^{mild}). ◩ refers to classical 21-hydroxylase deficiency haplotype (21-OH def^{severe}).

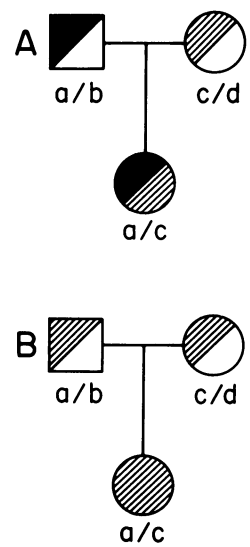

FIG. 2.—Possible 21-hydroxylase deficiency genotypes of heterozygous, unaffected parents with nonclassical offspring. See figure 1 for key to symbols.

TABLE 2

HLA GENE FREQUENCY ANALYSIS IN NONCLASSICAL AND CLASSICAL PATIENTS BY ETHNIC GROUPS

A. HLA gene frequency analysis in nonclassical patients by ethnic groups

POPULATION	No. HAPLOTYPES	GENE FREQUENCY					
		B14	*Bw47*	*B35*	*B8*	*B5*	*B40*
Controls[a]:							
1. Ashkenazi Jewish	254	.120[b]	.004	.150	.043	.085	.020
2. Hispanic	444	.033	.003	.051	.044	.144	.039
3. Yugoslav	152	.007	.040	.166	.090	.090	.011
4. Italian	1,044	.037	.004	.169	.062	.143	.023
Sum of ethnic control groups 1–4	1,894	.045	.006	.138	.058	.131	.025
Nonclassical patients[c]:							
1. Ashkenazi Jewish	56	.696[d]	0	.071	.018	.089	.018
2. Hispanic	9	.444[d]	0	0	0	.111	.111
3. Yugoslav	8	0	.125[e,f]	.125	0	.125	0
4. Italian	12	.667[d]	0	0	.083	0	.167[g]
5 "Other Caucasians"	17	.353	.176[e]	0	0	.059	0
Sum of ethnic patient groups 1–4	85	.600[d]	.012	.059[h]	.024	.082	.047

B. HLA gene frequency analysis in classical patients by ethnic groups

Controls (see above):							
American black	368	.039	.039	0	.011	.116	.027
Sum of all controls (plus North American Caucasians)	2,670	.040	.006	.122	.059	.119	.033

Total $< \frac{SW}{SV}$

Classical patients[i]:							
1. Ashkenazi Jewish	$12<\frac{6}{6}$	.167	0	.417[h]	0	.333[g]	0
2. Hispanic	$23<\frac{14}{9}$	0	0	.087	.043	.261	.043
3. Yugoslavs	$32<\frac{16}{16}$	0	.063	.219	.031	.125	0
4. Italian	$102<\frac{39}{63}$	.049	.010	.186	.010[g]	.157	.059[h]
5. "Other Caucasians"	$100<\frac{66}{34}$	.010	.100	.130	.050	.060	.130
6. Black	$11<\frac{9}{2}$	0	.091	.091[h]	0	.273	0
All classical (CAH)[j]	324	.025	.059[d]	.164[h]	.031[h]	.130	.080[d]
Salt-wasters	176	.006[g]	.085[d]	.170	.028	.108	.114[d]
Simple virilizers	148	.047	.027[g]	.155	.034	.155	.041

[a] From Baur and Danilovs, in *Histocompatibility Testing 1980* [35], p. 959.

[b] B. Dupont, unpublished data, 1984. New York City Ashkenazi *B14* frequency is the same as the worldwide Ashkenazi *B14* frequency.

[c] Ethnic background was homogeneous in each category and was compared to ethnically matched controls. The group "other Caucasians" includes non-Jewish persons of German, French, Polish, Russian, Hungarian, Greek, Anglo-Saxon, and Nordic origin (41% had at least one Anglo-Saxon ancestor). Significance is not reported for "other Caucasians" for lack of an appropriate control group. Haplotypes known to be associated with a classical 21-hydroxylase deficiency gene were omitted from this analysis, which explains the odd number of haplotypes.

[d] Underrule denotes statistical significance. $P \ll .001$.

[e] In these families, there were no known patients with the classical disorder; thus, the nonclassical patients might be genetic compounds carrying both a classical and nonclassical gene, but this could not be proven. The *HLA Bw47;DR7* segment was never associated with a proven nonclassical gene.

[f] Occasionally a *Bw47* variant is seen in association with *DR2*.

[g] Underrule denotes statistical significance. $P < .025$.

[h] Underrule denotes statistical significance. $P < .05$.

[i] Ethnic background was homogeneous in each category and was compared with ethnically matched controls. The group "other Caucasians" included non-Jewish persons of German, French, Polish, Russian, Hungarian, Greek, Anglo-Saxon, and Nordic origin (48% had at least one Anglo-Saxon ancestor). Significance is not reported for "other Caucasians" for lack of an appropriate control group. If the ethnic background was nonhomogenous (e.g., Italian-Polish), that haplotype was excluded from the analysis, which explains the odd number of haplotypes.

[j] This classification includes additional patients who are of mixed ethnic origin. The "sum of all controls (plus North American Caucasians)" served as a control.

TABLE 3

RELATIVE RISK

PATIENT GROUP	HLA-B ANTIGENS					
	B14	Bw47	B35	B8	B5	B40
Nonclassical:						
1. Ashkenazi Jewish	17.1	0	0.4	0.4	1.0	0.9
2. Hispanic	22.9	0	0	0	0.7	3.1
3. Yugoslavs	0	3.5	0.7	0	1.4	0
4. Italian	51.5	0	0	1.4	0	8.5
5. "Other Caucasians"	18.7	0	0	0	0.8	0
Sum of above ethnic groups 1–4*	31.9	1.9	0.4	0.4	0.6	1.9
Classical:						
1. Ashkenazi Jewish	1.5	0	4.0	0	5.3	0
2. Hispanic	0	0	1.7	0	2.1	1.1
3. Yugoslavs	0	1.6	1.4	0.3	1.4	0
4. Italian	1.3	2.6	1.3	0.15	1.0	2.6
5. "Other Caucasians"	0.1	22.7	2.0	0.8	0.7	3.2
6. Black	0	0	1.8	0	2.5	0
All classical†	0.6	10.3	1.5	0.5	1.2	2.4
Salt-wasting	0.1	15.3	1.5	0.4	0.9	3.7
Simple virilizing	1.2	4.7	1.4	0.6	1.4	1.3

NOTE: Underlined values are significant at least to the level of $P < .05$. Significance is not reported for "other Caucasians" for lack of an appropriate control group. Please see table 2*A* and *B* for composition of this group.

* The "sum of above ethnic groups 1–4" served as a control.

† The "sum of all controls (plus North American Caucasians)" served as a control.

either the classical or nonclassical gene, as the child could be either a compound heterozygote 21-OHdef^{severe}/21-OHdef^{mild} (fig. 2*A*) or a homozygote for the mild deficiency 21-OHdef^{mild}/21-OHdef^{mild} (fig. 2*B*) [20]. Results of the ACTH test do not distinguish between parents who have the genotypes 21-OHdef^{severe}/21-OH^{normal} and 21-OHdef^{mild}/21-OH^{normal}.

If, as indicated above, the parent on hormonal testing with ACTH proves to be a patient with nonclassical 21-hydroxylase deficiency, then that parent could be a compound heterozygote or a homozygote for the mild deficiency [20]. Figure 3 demonstrates the possible 21-hydroxylase deficiency genotypes of parents and offspring in families in which both a child and a parent have been diagnosed as patients with nonclassical 21-hydroxylase deficiency upon hormonal testing. In these families, there are no classical patients who allow us to identify the parental *HLA* haplotype linked to a classical genetic defect. In possibilities *A*, *B*, and *C* of figure 3, the haplotype of the affected parent not transmitted to the proband must carry the nonclassical gene, while in possibilities *D* and *E*, the haplotype of the affected parent not transmitted to the proband could carry a classical gene. We excluded the affected parents of offspring with nonclassical 21-hydroxylase deficiency from this analysis to avoid the possible error shown in figure 3*D* and *E* unless the haplotype not transmitted to the proband also carried *HLA-B14*, which we document here to be in genetic linkage disequilibrium with the nonclassical 21-hydroxylase deficiency gene. This exception applied to three Ashkenazi Jewish families. In these families, there was a high probability that the haplotype not transmitted to the affected offspring carried a nonclassical genetic defect. Two additional Ashkenazi Jewish families were included although the haplotype not transmitted to the affected offspring carried B40 and B8 antigens, respectively, because these anti-

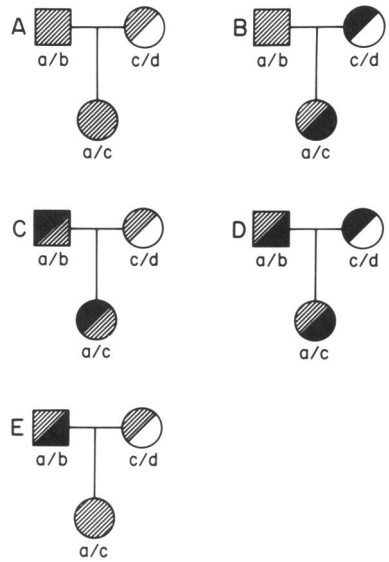

FIG. 3.—Possible 21-hydroxylase deficiency genotypes in families in which both an offspring and a parent have been diagnosed as patients with nonclassical 21-hydroxylase deficiency upon hormonal testing. See figure 1 for key to symbols.

gens, which are not found in any Ashkenazi classical patients, were present in some nonclassical Ashkenazi patients.

The occurrence of nonclassical 21-hydroxylase deficiency in these parents was detected by hormonal criteria using the nomograms referred to above [28]. By counting the incidence of nonclassical 21-hydroxylase deficiency in parents, we could estimate the frequency of the nonclassical deficiency gene relative to the presumed normal genes (table 4).

Thus, for example: (1) There were 94 parental haplotypes in our Ashkenazi Jewish families. (2) Of these 94, 47 are obligate carrier haplotypes and the other 47 haplotypes represent, a priori, a random sample of (normal and 21-hydroxylase-deficient) haplotypes in the population. (3) Among the parents, we found nine who were actually nonclassical patients rather than heterozygotes upon hormonal testing. (4) Therefore, the gene frequency, q, for the nonclassical 21-hydroxylase gene is estimated as: 9 nonclassical genes/47 random genes = .191 or 1/5 (95% confidence limits: .092–.333). (5) Heterozygote frequency = $2pq$ = 2(.191)(.809) = .309 or approximately 1/3. (6) Nonclassical disease frequency = q^2 = .037 or 1/27 by Hardy-Weinberg Law for a population at equilibrium [40] (95% confidence limits .008–.111, or 1/9 to 1/125). This analysis was carried out for each ethnic group studied.

II. Sib Pair Analysis

This method of analysis was used to confirm the above method. *HLA* genotypes of sib-pairs in families with two or more similarly affected members in 1 generation were analyzed according to the method quoted by Thomson and Bodmer [41]. Among the families with nonclassical 21-hydroxylase deficiency, 14 of 18 sib-pairs were *HLA*-identical. Among the four sib-pairs who were not identical, one sib-pair shared no *HLA* haplotype and three sib-pairs shared one haplotype. (1) 3 (affected sib pairs shared only one haplotype)/18 (total number of sib pairs) = .167. (2) By reference to table 2 in Thomson and Bodmer [41], .167 yields a nonclassical gene frequency of .1 or 10% (95% confidence limits are approximately .009–.324). (3) Heterozygote frequency: $2pq$ =

TABLE 4

NONCLASSICAL 21-HYDROXYLASE (NC 21-OHD) GENE AND DISEASE FREQUENCIES

A. Hormonal criteria*

ETHNIC GROUP†	PARENTAL HAPLOTYPES: Total no.	PARENTAL HAPLOTYPES: No. random	NO. RANDOM PARENTAL HAPLOTYPES NONCLASSICAL 21-OHD BY ACTH	GENE FREQUENCY (q)	DISEASE FREQUENCY (q^2) (95% CONFIDENCE LIMITS)	HETEROZYGOTE FREQUENCY [$2q(1 - q)$]
Ashkenazi Jewish	94	47	9	.191 (.092–.333)	.037 (.008–.111)	.309 (.166–.445)
Hispanic	44	22	3	.136 (.029–.349)	.019 (.001–.122)	.235 (.056–.454)
Yugoslavs	80	40	5	.125 (.042–.268)	.016 (.002–.072)	.219 (.080–.392)
Italian	208	104	6	.058 (.022–.121)	.003 (.0005–.015)	.109 (.042–.213)
"Other Caucasians"†	112	56	2	.036‡ (.004–.123)	.001 (.00002–.015)	.069 (.009–.216)
Black	14	7	0	‡		
American Indian	4	2	0	‡		
Sum of above ethnic patient groups	556	278	25	.090 (.059–.130)	.009 (.003–.017)	.164 (.111–.226)

B. Sib-pair analysis

	Total haplotypes	Sib-pairs sharing 1 *HLA* haplotype	q [41]	q^2	$2q(1 - q)$
Mixed ethnic groups§	18	3 (.648–7.452)	.100 (.009–.324)	.010 (.00008–.105)	.180 (.018–.438)

* Only parents who had undergone ACTH testing were included.

† Ethnic background was homogenous in each category, except "other Caucasians," which includes non-Jewish persons of German, French, Polish, Russian, Hungarian, Greek, Anglo-Saxon, and Nordic origin.

‡ Because of low disease frequency in these ethnic groups, more families must be studied.

§ Including Ashkenazi Jews, Anglo-Saxons, Italians, Hispanics, Germans, and Native American Indians.

2(.1)(.9) = .18 or 1/5 to 1/6 people. (4) Expected nonclassical disease frequency: q^2 = .01 or 1/100 (95% confidence limits are .00008–.105).

III. Association with HLA-B14

Linkage disequilibrium between *HLA-B14* and the gene for nonclassical 21-hydroxylase deficiency was analyzed for each ethnic group (table 5*A*) by studying the parental haplotypes not transmitted to the affected offspring. As in the hormonal test, this haplotype was considered a random parental haplotype. The raw data were arrayed in two-by-two contingency tables (table 5*A*) that allowed us to analyze the genetic association of nonclassical 21-hydroxylase deficiency with *HLA-B14*, using the statistics indicated in table 5*B* derived from Thomson and Bodmer [41].

RESULTS

Association between HLA-B *and Classical and Nonclassical 21-Hydroxylase Deficiency*

Nonclassical Patients

Analysis of the *HLA* typing data showed a highly significant increase in the frequency of *HLA-B14* among all nonclassical patients in each ethnic group studied, except Yugoslavs, when compared with ethnically matched controls (table 2*A*). The highest gene frequency for *B14* was exhibited by the Ashkenazi Jewish nonclassical patient group (69.6%, $P \ll .001$) compared with ethnically and geographically matched controls (12%). *HLA-B14* was associated with *DR1* in 28 of 29 Ashkenazi Jewish haplotypes and in 45 of 49 haplotypes in the overall nonclassical population where both HLA-B and -DR typing was performed. Further, *B35* was significantly decreased in all nonclassical ethnic patient groups except Yugoslavs. The other notable association with the nonclassical gene is the increased frequency of *HLA-B40* in Italians. The fact that the decrease in the frequency of *HLA-B8* did not achieve overall statistical significance among nonclassical patients reflects the relatively high frequency of *B8* in patients of Italian origin.

Classical Patients

In contrast to the nonclassical patients, *B14* was not increased in classical patients. However, *Bw47* was increased to a significant degree in classical patients from all groups (table 2*B*). Forty-two percent of the patients with *Bw47* were of Anglo-Saxon origin, while no Ashkenazi Jewish patient carried the *Bw47* antigen. *HLA-Bw47* was associated with *DR7* in 16 of 16 haplotypes where both HLA-B and -DR typing was performed. *B35* and *B5* were significantly increased in Ashkenazi Jewish families but not strikingly increased among other ethnic groups. *B40* was significantly increased in patients of Italian origin. *HLA-B40* was also increased in all groups of classical patients, a consequence of the high *B40* frequency in salt wasters, while *B8* was decreased, confirming our previous report [29].

Relative Risks

The relative risk (table 3) for nonclassical disease with the B14 antigen was 31.9 overall, while it was only 1.9 for Bw47. There was a low risk of both

TABLE 5

GENETIC ASSOCIATION BETWEEN HLA-B14 AND NONCLASSICAL 21-HYDROXYLASE DEFICIENCY

A. Raw data: analysis of parental haplotypes not transmitted to offspring with either classical or nonclassical 21-hydroxylase deficiency distributed according to their HLA type and ethnic group

	B14 +	B14 −	Row total	
Ashkenazi Jewish:				
Haplotype without 21-OH deficiency gene ..... a	3	c 33	36	
Haplotype with 21-OH deficiency gene ..... b	6	d 3	9	
Column total	9	36	45	= N = Grand total
Hispanic:				
Haplotype without 21-OH deficiency gene	0	18	18	
Haplotype with 21-OH deficiency gene	2	1	3	
Column total	2	19	21	
Yugoslav:				
Haplotype without 21-OH deficiency gene	0	32	32	
Haplotype with 21-OH deficiency gene	0	4	4	
Column total	0	36	36	
Italian:				
Haplotype without 21-OH deficiency gene	3	90	93	
Haplotype with 21-OH deficiency gene	5	0	5	
Column total	8	90	98	
"Other Caucasians"				
Haplotype without 21-OH deficiency gene	1	52	53	
Haplotype with 21-OH deficiency gene	1	1	2	
Column total	2	53	55	
Sum of above ethnic groups:				
Haplotype without 21-OH deficiency gene	7	225	232	
Haplotype with 21-OH deficiency gene	14	9	23	
Column total	21	234	255	

Table 5 continued on next page

TABLE 5 *(continued)*

B. Analysis of linkage disequilibrium by ethnic group

	P_A	P_D	P_{AD}	D	D/P_{AD}	*P*	x ± SE
Ashkenazi Jewish	.083	.200	.133	.093	.699	<.001	.667 ± .158
Hispanic	0	.143	.095	.082	.863	<.014	1.000
Yugoslav	0	.111	...	...	...	...	...
Italian	.032	.051	.051	.047	.922	<.0001	.625 ± .171
Other Caucasian	.019	.038	.018	.017	.937	<.09	.500 ± .354
Sum of above ethnic groups	.031	.090	.055	.047	.833	<.0001	.667 ± .103

NOTE: The designations a, b, c, and d refer to the cells of the 2 × 2 contingency tables as indicated in table 5*A*. $N = a + b + c + d$. $P_A = a/(a + b)$ = frequency of B14 in 21-hydroxylase normal haplotypes not transmitted to affected offspring. $P_D = (c + d)/N$ = frequency of the nonclassical 21-hydroxylase deficiency in haplotypes not transmitted to the affected offspring. $P_{AD} = c/N$ = frequency of the haplotype containing both nonclassical 21-hydroxylase deficiency and HLA-B14 alleles. $D = |bc - ad|/N^2$ = linkage disequilibrium between B14 and the nonclassical 21-hydroxylase deficiency gene. D/P_{AD} = linkage disequilibrium standardized for the frequency of the haplotype containing both the nonclassical 21-hydroxylase deficiency and HLA-B14 alleles. *P* = level of significance of linkage disequilibrium between B14 and the nonclassical 21-hydroxylase deficiency gene calculated from the 2 × 2 tables (Fisher exact method). x = c/(a + c) = penetrance of the nonclassical 21-hydroxylase deficiency gene in B14-containing haplotypes. SE = standard error.

classical and nonclassical disease with the B8 antigen.The relative risk for nonclassical disease was less than one for subjects with B5 and B35, and slightly greater than one for the B40 antigen. Table 3 also gives the relative risks of classical 21-hydroxylase deficiency. For the Bw47 antigen, it was 4.7 in the case of the simple virilizing form and 15.3 in the salt-wasting form. The antigens B5, B35, and B40 were associated with small increases in relative risk for classical disease, while B14 showed a reduced risk.

Nonclassical 21-Hydroxylase Deficiency Gene Frequency

I. *Hormonal analysis (table 4).* Analysis of hormonal data in Ashkenazi Jewish families yielded a nonclassical 21-hydroxylase deficiency gene frequency of .191 and a disease frequency of 1/27 derived from Hardy-Weinberg Law. In Hispanic and Yugoslav subjects, the disease frequencies were 1/53 and 1/63, respectively. Italian subjects showed a disease frequency of 1/333. The lowest frequency was found among the subgroup of "other Caucasians" (1/1,000), which includes many Anglo-Saxons. For the total combined ethnic groups, the disease frequency was 1/111. The heterozygote frequency ranged from a high of approximately 1/3 in Ashkenazi Jews to a low of 1/14 in "other Caucasians." Ninety-five percent confidence limits for gene, disease, and heterozygote frequencies are given in table 4.

II. *Sib-pair analysis.* Gene-frequency analysis by the sib-pair method for closely linked recessive disease susceptibility genes [41] is based on the principle that as the disease gene becomes increasingly rare, a greater proportion of affected sib-pairs is expected to be *HLA*-identical. Thus, the proportion of sibs sharing both, one, and zero haplotypes allows calculation of the gene frequency in the population. Among classical patients, 30 of 30 sib-pairs were *HLA*-identical, whereas among nonclassical patients, only 14 of 18 sib-pairs were

HLA-identical. The frequency of nonclassical 21-hydroxylase deficiency disease calculated as discussed by Thomson and Bodmer [41] was 1/100 (see SUBJECTS, MATERIALS, AND METHODS above).

By both methods of calculation, the frequency of nonclassical disease is at least 1/100 in the Ashkenazi Jewish population and is also very high in other populations (table 4).

III. *Linkage disequilibrium* (table 5*A* and *B*). Linkage disequilibrium between *HLA-B14* and the gene for nonclassical 21-hydroxylase deficiency was statistically significant in Ashkenazi Jews, Italians, and Hispanics; the sample size was small, however, in the latter group. Genetic linkage disequilibrium was not significant in the group of "other Caucasians." In the Yugoslavs, linkage disequilibrium could not be calculated because *B14* did not occur in any haplotype not transmitted to the proband. The mixed ethnic group also showed significant linkage disequilibrium, but this was an obvious consequence of the high values in the Ashkenazi Jewish, Italian, and Hispanic populations. The linkage disequilibrium remained surprisingly high and homogeneous after the values were standardized for the frequency of the haplotype containing both *B14* and nonclassical 21-hydroxylase deficiency (D/P_{AD}).

Finally, the penetrance (as defined in table 5*B*) of the hypothetical nonclassical 21-hydroxylase deficiency gene is not complete, its average estimate being .667 ± .103. Although this figure is obviously low for monofactorial genetic traits, it is of the order of magnitude found in several studies of *HLA*-associated disease: in ankylosing spondylitis, it has been estimated as .38 ± .06 [42], which is not statistically different from our findings. Additionally, the variable expressivity of the clinical condition contributes to this phenomenon.

DISCUSSION

In this study, we have documented that nonclassical 21-hydroxylase deficiency is probably the most frequent autosomal recessive genetic disorder in man (fig. 4), clearly evident in the overall Caucasoid population. When groups of different ethnic background were analyzed separately, the nonclassical 21-hydroxylase deficiency occurred with higher frequency in Ashkenazi Jews, Hispanics, and Yugoslavs than in the other ethnic groups. It would be desirable to increase the size of each ethnic group studied in order to narrow the confidence limits so that one might ascertain whether any ethnic group has a statistically higher frequency of the disorder than another. A routine screening test at the population level, currently not available, would be a valuable means of increasing the patient sample. Although the microfilter method can detect elevated serum 17-OHP levels in the patients with the classical disorder, it is not sufficiently sensitive to detect the lower serum 17-OHP levels observed in the nonclassical disorder [12]. Since the ACTH test is burdensome, we have therefore relied on analytical family studies for the analysis herein.

Establishing the precise disease prevalence in each ethnic group is potentially important in understanding the origin and the currently extremely high frequency of the nonclassical 21-hydroxylase deficiency mutation. In general, the high frequency of a mutation such as nonclassical 21-hydroxylase deficiency is attributable to genetic drift or to variation in fitness, and the

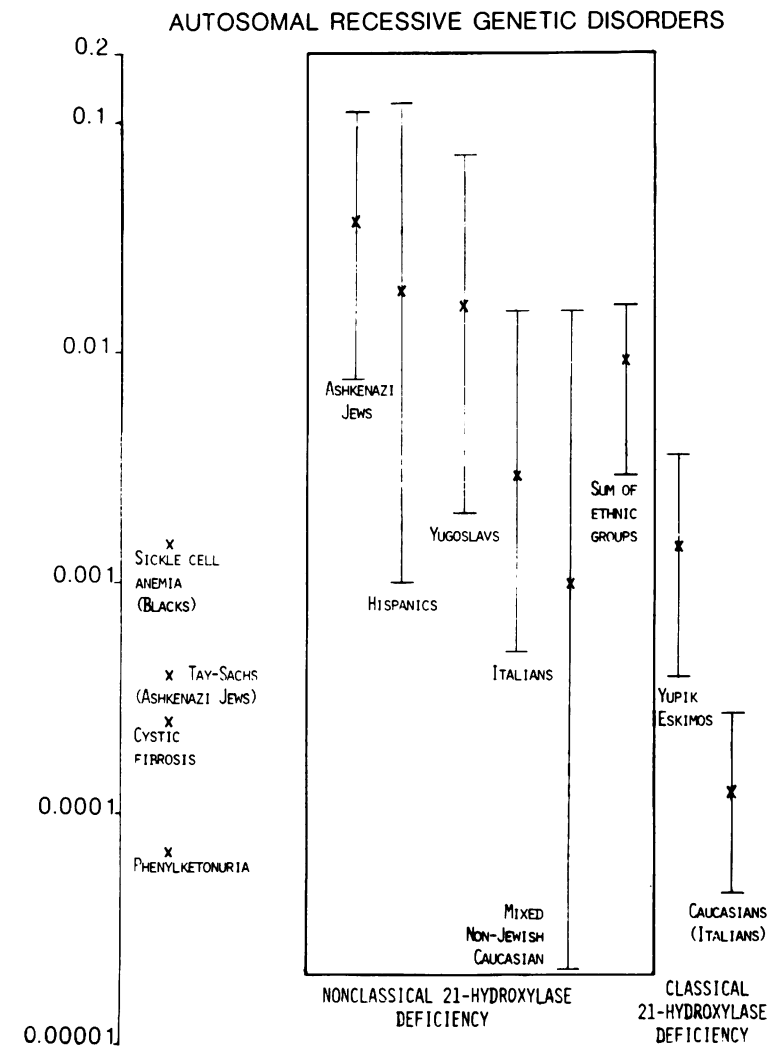

FIG. 4.—The disease frequencies of nonclassical 21-hydroxylase deficiency and classical 21-hydroxylase deficiency relative to other common autosomal recessive disorders. The latter frequencies derived from Behrman et al. [43]. Bars represent the 95% confidence limits.

discrimination between these is all the more interesting because of the linkage disequilibrium with the *HLA* haplotype. Significant disequilibrium between alleles at two tightly linked loci may have been fixed by chance, which is most probable for groups like Jewish people, who have undergone many abrupt changes in population size and geographic distribution. If the mutation affected a *B14*-carrying haplotype, for example, this haplotype may have increased in frequency through founder effects and sufficient time would not yet have elapsed to allow for the achievement of equilibrium. Alternatively, if the similarities in the disease frequency and *HLA* association among different

ethnic groups are borne out by analysis of larger samples, selective influences underlying the maintenance of the linkage disequilibrium might be a likely explanation. Since the clinical syndrome resulting from the homozygous state may include infertility in both sexes, some sort of selective advantage for the heterozygote that outweighs this reduced fertility appears to be necessary for the maintenance of the high disease prevalence.

A decreased frequency of *B35* and *B8* was noted in all groups of Caucasoid patients with nonclassical 21-hydroxylase deficiency except Yugoslavs and Italians. The implications of these negative associations are currently under study [9]. One might speculate as to whether there is some "protective" effect conferred by these *HLA-B* associations.

The linkage disequilibrium between *HLA-B14* and the gene for nonclassical 21-hydroxylase deficiency in Ashkenazi Jews, Italians, and Hispanics suggests that the nonclassical 21-hydroxylase deficiency mutations might be the same in these populations and different from that of the classical 21-hydroxylase deficiency, which is in association with *Bw47*. Also, the mutation in Yugoslav patients with nonclassical 21-hydroxylase deficiency, which is not in association with *HLA-B14* (as also found by Brkljacic et al. [44]), may have an independent origin from that found in Ashkenazi Jewish, Italian, and Hispanic patients. This question might be resolved when the mutation is studied at the molecular level.

Bonne-Tamir [45] reported that *B14* is specifically increased among Ashkenazi Jewish families and not among Sephardic and Oriental Jews. Since *B14* is found in linkage disequilibrium with the nonclassical 21-hydroxylase deficiency gene, the mutation probably occurred during the early second millenium, A.D., at which time world Jewry was consolidated into German-Polish (Ashkenazi), Spanish (Sephardic), and Near Eastern (Oriental) communities.

The high frequency of the 21-hydroxylase deficiency must be taken in the context of the linkage disequilibrium maintained by many HLA-A, B, C, and D antigens. The classical 21-hydroxylase deficiency gene has been mapped in the region adjacent to the *C4* complement locus between *HLA-B* and *D* [9]. Recent studies have specified the defect at the DNA level in patients with the classical 21-hydroxylase deficiency and HLA-Bw47 [8]. In normal subjects, there appear to be two 21-hydroxylase genes near the two *C4* complement genes, while in patients with classical 21-hydroxylase deficiency linked to *HLA-Bw47*, one of these 21-hydroxylase genes is deleted [8, 9]. Selective defects in expression of HLA class I (i.e., *HLA-A, -B, -C*) region genes and *HLA* class II (i.e., *HLA-DR, -DQ, -DP*) region genes have not been described. In contrast, selective defects in the expression of *HLA*-class III region genes are quite common. For example, selective defects in expression of complement C4A (Rodgers antigen) occurs in 2% of the population and selective defects in expression of C4B (Chido antigen) occurs in 5% of the population [46–48]. We now describe that selective defects in expression of 21-hydroxylase resulting in late-onset 21-hydroxylase deficiency occurs in 1% of the population, while classical 21-hydroxylase deficiency occurs in .01%–.02% [11]. These findings indicate that the *HLA*-class III region probably contains a chromosomal segment that is subject to frequent mutation (i.e., mutational "hot spots").

Molecular genetic analysis of the *C4/21-hydroxylase* region suggests that mutations in this region resulting in deletions or gene duplications could cause sufficient misalignment of chromosomes to reduce the likelihood of recombination [9]. This could be responsible for an apparent suppression of recombination within the *HLA* region and thereby provide a mechanism for maintenance of positive genetic linkage disequilibrium between *HLA* alleles at different loci. Future studies at the molecular level are obviously required to specify the genetic defect in the nonclassical form of steroid 21-hydroxylase deficiency.

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