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Human Neutral α-Glucosidase C: Genetic Polymorphism Including a "Null" Allele

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SUMMARY

We describe a genetic polymorphism of human neutral α-glucosidase C, detected in lymphoid cells by a combination of starch gel electrophoresis and isoelectric focusing. The seven phenotypes observed appear to result from the expression of four different alleles. The distribution of the observed phenotypes fits the expected distribution predicted from calculated gene frequencies in Hardy-Weinberg equilibrium. Family studies are consistent with autosomal inheritance of the gene. The product of one of the alleles is unusual in that it is "silent," with an estimated gene frequency of .174 in an outbred white population. Approximately one-third of the population is heterozygous "null." Homozygosity for the allele has not been associated with any obvious disease state. This is the third example of a "null" allele which has a substantial gene frequency in an outbred population but does not appear to result in disease in the homozygous state.

INTRODUCTION

Human α-glucosidases can be identified by means of electrophoresis in starch gel of tissue extracts and subsequent staining (at various pHs) with the artificial substrate 4-methylumbelliferyl-α-D-glucopyranoside (4-MUF). Most tissues (lymphoid-line cells, liver, thymus, tonsil, fibroblasts, and peripheral blood lymphocytes) exhibit two major forms of enzyme activity when stained at neutral pH: neutral α-glucosidase AB (α-Glu AB), and neutral α-glucosidase C (α-Glu_C). Neutral α-Glu AB, which appears to

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consist of two related isozymes, differing in the degree of sialation, migrates most rapidly to the anode. Neutral α-Glu_C migrates less rapidly to the anode and is not affected by treatment with neuraminidase [1]. A third band of neutral α-glucosidase activity with even slower mobility toward the anode is seen upon electrophoresis of kidney extracts. However, under the conditions of starch gel electrophoresis, this isozyme is not seen in extracts of lymphoid cells (Martiniuk and Hirschhorn, unpublished data, 1980). Lysosomal acid α-glucosidase, its deficiency resulting in Pompe disease (type II, glycogen storage disease), is identified only after staining at acid pH and usually has the slowest mobility toward the anode [1].

In studying the isozymes of α-glucosidase, we found an inherited polymorphism of the neutral α-Glu_C. Comparing the results of starch gel electrophoresis at two different pHs and isoelectric focusing (IEF), we have distinguished seven different phenotypes produced by four alleles, one of which is silent.

MATERIALS AND METHODS

Lymphocyte Separation

Peripheral blood lymphocytes were separated from 15 ml of anticoagulated (EDTA or heparin) whole blood by centrifugation on sodium metrizoate/ficoll density gradients by the method of Boyum [2]. The PBS-washed cell pellets were stored at $-60°$ until use. A minimum of 8×10^6 cells suspended in 50 μl are needed for obtaining sharp bands of enzyme activity in the three typing gels.

Long-term lymphoid lines were obtained from the Division of Human Genetics, Mount Sinai School of Medicine and from the National Institutes of Health Human Genetic Mutant Cell Repository, Camden, New Jersey.

Sample Preparation

The peripheral blood lymphocytes obtained from 15 ml of blood were suspended in 50 μl H_2O, transferred to 400 μl microfuge tubes, and sonicated three times (5 seconds per burst) (Heat Systems-Ultrasonics, Plainview, N.Y.). The extract was then centrifuged for 5 min at 4°C in a Beckman microfuge and the supernatant used for electrophoresis.

Starch Gel Electrophoresis

Electrophoresis was carried out in a support of 10.1% hydrolyzed starch gel (Connaught Laboratories, Toronto, Canada) in 0.01 M sodium phosphate, pH 6.5 or pH 7.0, at 10 V/cm for 30 min followed by 11.5 V/cm for 4½ hrs on cooling plates kept at 4°C. Extracts (15 μl) were applied to Whatman 3 mm paper inserts and electrophoresed [1]. The gel was stained by overlaying with Whatman 17 chromatographic paper saturated with 4-MUF (0.5 mg/ml, dissolved by briefly heating in a small volume of ethanol and then diluted in 0.1 M sodium phosphate, pH 7.5) and incubated at 37°C. Enzyme activity was visualized as fluorescent bands under long-wave UV light. On occasion, NH_4OH was sprayed on the gel to increase the intensity of the fluorescent bands.

IEF

Horizontal slab IEF was performed on 5% polyacrylamide focusing gel containing a final concentration of 8mg% riboflavin with a pH gradient of 4–8, which was linear from pH 5–7, essentially as described in LKB (Rockville, Md.) application note 75. However, the concentrations of ampholytes were modified to contain 0.3 ml each of the pH 4–6 and 7–9 and 2.4 ml of the pH 5–7 LKB ampholytes. The gel was polymerized under UV light for 60 min and stored at 4°C until use (less than 1 week). IEF was carried out on an LKB multiphor electrophoresis apparatus at 4°C. The gel was prefocused for 60 min with a stepwise increase of voltage to

700 V. A 20 μl sample (same sample as for starch gel electrophoresis) was applied with an Eppendorf pipette as a 1.0 cm streak, approximately 1–2 cm from the negative electrode wick. The gel was focused for 4 hrs at 1,000 V, after which enzyme activity was detected by overlaying with Whatman 17 chromatographic paper saturated with the 4-MUF stain. The gel was incubated for 20 min at 37°C, the paper overlay removed, and NH_4OH-saturated, 3-mm Whatman paper placed on the gel for 30 seconds to increase the intensity of the fluorescent bands seen at the site of enzyme activity. The NH_4OH-saturated paper was removed and the enzyme bands visualized under UV light. (This must be done as fast as possible to stop diffusion.) The isoelectric point (pI) of neutral α-Glu_C-1 was initially determined using partially purified material, and only the neutral α-Glu_C activity is visualized under these conditions (Martiniuk and Hirschhorn, unpublished data, 1980).

RESULTS

Starch gel electrophoresis of cultured human lymphoid-line cell extracts from different individuals revealed mobility differences of the α-Glu_C isozyme. These differences in mobility were characteristic for individual cell lines and were not altered following incubation with 2-mercaptoethanol, dithiothreitol, neuraminidase, or storage at 4°C. The differences in mobility of α-Glu_C were not accompanied by mobility differences of the neutral α-Glu AB isozymes. In most cases (43 of 58 lines), a single slow band of enzyme activity, α-Glu_C-1, was visualized after electrophoresis of lymphoid-line extracts on starch gel at pH 6.5 (table 1, fig. 1). A single, slightly faster band, α-Glu_C-2, was visible in three of 58 lines, and an even faster band, α-Glu_C-3, in two of 58 lines. The ten remaining lymphoid lines each exhibited two bands consisting of all the possible combinations, that is, α-Glu_C-2-1, 3-1, and 3-2. The absolute mobility of the fastest isozyme (α-Glu_C-3)—but not of α-Glu_C-1 or α-Glu_C-2—was found to vary to some extent with the concentration of the extract. More concentrated samples of α-Glu_C-3 had the greatest anodal mobility, whereas serially diluted samples showed progressively slower mobility. As a result of this variation, α-Glu_C-3 was, on occasion, indistinguishable from α-Glu_C-2, and repeated electrophoresis of samples of varying strength was sometimes required for reliable typing.

To distinguish more clearly between the phenotypes α-Glu_C-2 and α-Glu_C-3, or combinations involving their alleles, we performed starch gel electrophoresis at pH 7.0 and IEF in acrylamide gels (pH 5–7 range). Unexpectedly, the α-Glu_C-3 band, as

TABLE 1

NO. INDIVIDUALS OF DIFFERENT NEUTRAL α-GLU_C PHENOTYPES AS DETERMINED BY ELECTROPHORESIS AND IEF FOR LONG-TERM LYMPHOID CELLS AND PERIPHERAL BLOOD LYMPHOCYTES

		PHENOTYPE							
	POPULATION	1	2-1	2	3-1	3	3-2	0	TOTAL
Lymphoid lines	Unknown	43	5	3	4	2	1	0	58
Peripheral blood lymphocytes	White	69	14	5	3	0	0	3	94
	Hispanic	15	3	2	3	0	0	0	23
	Black	5	18	2	2	0	1	0	28
	Oriental/Eastern Indian	9	1	2	2	1	0	0	15

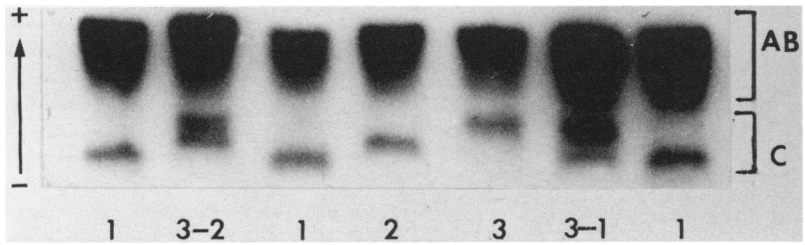

FIG. 1.—Starch gel electrophoresis at pH 6.5. Five of seven phenotypes of neutral α-Glu_C observed are illustrated. Single bands of neutral α-Glu_C with three differing mobilities toward anode are found. Most commonly observed is a single band with the slowest mobility to anode (in *first*, *third*, and *seventh channels*), or α-Glu_C-1. Several extracts exhibit a single band migrating more rapidly to anode (*fourth channel*) or α-Glu_C-2. Finally, an even more rapidly migrating enzyme band is observed (*fifth channel*) or α-Glu_C-3. Double bands containing all possible combinations of the single bands were observed. Illustrated are α-Glu_C-3-1 (*sixth channel*) and α-Glu_C-3-2 (*second channel*). α-Glu_C-2-1 and α-Glu_C-0 are not shown. On occasion (see text), α-Glu_C-2 and α-Glu_C-3 can comigrate.

defined by starch gel electrophoresis at pH 6.5, comigrated with α-Glu_C-1 both on starch gel at pH 7.0 and on IEF, with a pI of 5.5 ± 0.2. In contrast, the α-Glu_C-2 band, as defined by electrophoresis at pH 6.5, still had greater anodal mobility than α-Glu_C-1 at pH 7.0 and had, as expected, a clearly lower pI (approximately 0.1 U) (figs. 1, 2, and 3). All phenotypes usually demonstrated an additional weaker, anionic band of enzyme activity, detectable only by means of IEF. IEF gave more clearly defined bands of enzyme activity and was, therefore, more reliable for typing than starch gel electrophoresis at pH 7.0.

We could, thus, reliably distinguish six different phenotypes (fig. 3), that is, α-Glu_C-1, α-Glu_C-2, α-Glu_C-3, and combinations of them, by comparing two starch gels (pH 6.5 and 7.0) and IEF of 58 lymphoid-line extracts. However, after calculating theoretical gene frequencies from the observed phenotype distribution, the observed frequencies did not fit those predicted by Hardy-Weinberg equilibrium for three alleles ($> .99$). Since we did not know the donors' race and sex for many of the lymphoid lines and since we found that several lines carried new chromosomal translocations, trisomies, etc., we surveyed peripheral blood lymphocytes from a normal population to eliminate these variables.

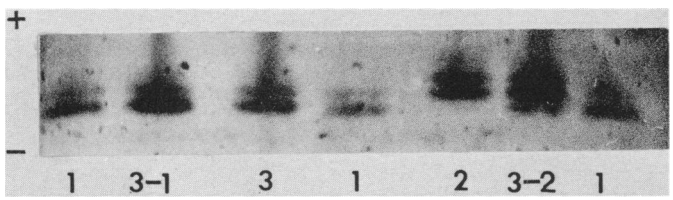

FIG. 2.—Horizontal slab polyacrylamide IEF was performed on same samples which were electrophoresed in starch gel (FIG. 1). All phenotypes usually demonstrated an additional weaker, more anionic band of enzyme activity on IEF. Extracts of α-Glu_C-3 (*third channel*) as defined by starch gel electrophoresis, pH 6.5, are indistinguishable from common α-Glu_C-1 (*first*, *fourth*, and *seventh channels*); in contrast, α-Glu_C-2 (*fifth channel*) has a clearly lower pI (0.1 U lower) and is easily distinguished from α-Glu_C-1 and, most importantly, from α-Glu_C-3. α-Glu_C-3 cannot be distinguished from α-Glu_C-1 or 3-1 on IEF. α-Glu_C-2-1 and 2-3 also cannot be distinguished from each other, but are clearly different from the other phenotypes on IEF and can be distinguished from each other by comparison with the results of starch gel electrophoresis.

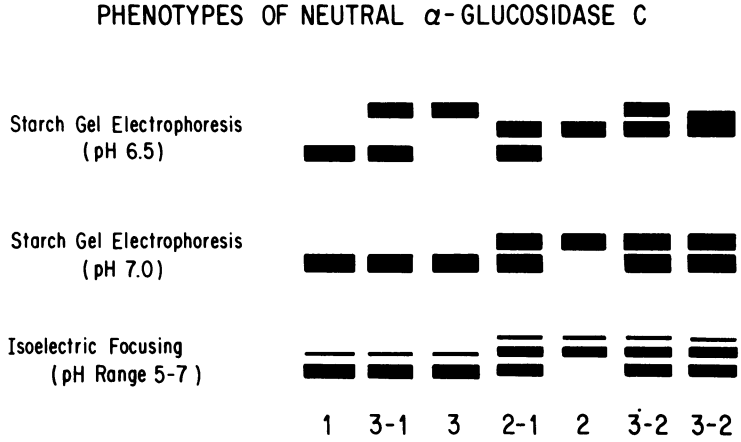

FIG. 3. —Representation of six of seven observed phenotypes for neutral α-Glu_C of long-term lymphoid lines or peripheral blood lymphocytes as determined by combination of starch gel electrophoresis, pH 6.5 and 7.0, and IEF. (Anode or lower, more acidic pI is at *top* of diagram). Product of the α-Glu_C^1 allele has the slowest anodal mobility on starch gel electrophoresis at both pH 6.5 and 7.0 and a higher, more basic pI. Product of the α-Glu_C^2 allele has a faster anodal mobility on starch gel and a lower pI than α Glu_C-1. Product of the α Glu_C^3 allele usually has the fastest anodal mobility on starch gel electrophoresis at pH 6.5 but can comigrate with α Glu_C-2. However, α Glu_C-3 (but not α Glu_C-2) comigrates with α Glu_C-1 in starch gel at pH 7.0 and has a pI indistinguishable from α Glu_C-1 but higher than α Glu_C-2 on IEF. Heterozygous states can similarly be defined by combined starch gel electrophoresis and IEF.

All of the phenotypes observed in lymphoid-line cells were also observed in peripheral blood lymphocytes of 160 individuals of different ethnic backgrounds (table 1). In addition, we found three of 94 white individuals whose lymphocyte extracts showed no enzyme activity for neutral α-Glu_C, despite exhibiting normal activity of the α-Glu AB isozyme. The α-Glu C isozyme was not detected even when 10 times more concentrated extracts were used. These three α-Glu_C-0 individuals were apparently healthy, young adult normal volunteers. We then considered the possibility that these extracts could contain an allozyme with greater increased anodal mobility which was obscured by the α-Glu AB isozymes still present. We, therefore, removed the α-Glu AB isozymes by incubating lysates from one such α-Glu_C-0 individual and from individuals with different α-Glu_C phenotypes with Con A-Sepharose, which binds the glycosylated α-Glu AB isozymes [3]. Electrophoresis of Con A-treated extracts, which no longer contained the AB isozymes, did not uncover any area of neutral α-Glu C activity in lysates from the "null" individual, but α-Glu C was still present in extracts from individuals of the other phenotypes. To detect neutral α-Glu C activity with an increased, as well as decreased, pI, extracts were subjected to IEF, that is, to conditions in which the α-Glu AB isozymes will not be identified and, hence, will not interfere with detection of α-Glu C activity. Neutral α-Glu C activity was also not observed in the extracts from the null individual at any pI from 4 to 8. Additional evidence for a null allele was provided by the results of quantitative assay. α-Glu C, but not α-Glu AB, uses glycogen as substrate, thus allowing for determination of α-Glu C activity in the presence of α-Glu AB [4]. When lymphoid-line cell extracts from a null individual were passed through an antiacid α-glucosidase antibody column

and then assayed for glycogenolytic activity at neutral (as well as acid) pH, no activity was observed. The sensitivity of the assay allows us to state that there is less than 5% of normal glycogenolytic activity. The presence of the AB isozymes in the absence of any α-Glu C isozyme in the null individuals also indicates that neutral α-Glu AB and α-Glu C are controlled by at least two separate genetic loci.

Because of the presence of the null (α-Glu_C^0) allele, the apparent homozygous phenotypes observed in the white population (α-Glu_C-1, 2, or 3) had to be considered differently. A certain proportion of the apparently homozygous individuals are, therefore, heterozygous for the α-Glu_C^0 allele, and calculations of the gene frequencies must take into consideration the fourth, null allele. We calculated the gene frequencies in the white population from the observed frequencies of the phenotypes α-Glu_C-2-1, 3-1, and α-Glu_C-0. If α-$Glu_C^1 = p$, α-$Glu_C^2 = q$, α-$Glu_C^3 = r$, and α-$Glu_C^0 = s$ and Hardy-Weinberg equilibrium holds true, then α-Glu_C-2-1 $= 2pq = 14/94 = .1489$; α-Glu_C-3-1 $= 2pr = 3/94 = .0319$; and α-Glu_C-0 $= s^2 = 3/94$, $s = .1786$. Therefore, $p = .0744/q$ and $r = .2143q$; substituting into $p + q + r + s = 1$, gene frequencies were calculated. The estimated gene frequencies were α-$Glu_C^1 = .690$, α-$Glu_C^2 = .108$, α-$Glu_C^3 = .023$, and α-$Glu_C^0 = .179$. An alternative calculation was performed using the observed phenotype frequencies of α-Glu_C-1, 2, and 0, again recognizing that the phenotypes α-Glu_C-1 and 2 included heterozygotes for the null allele. The gene frequencies were calculated from: α-Glu_C-0 $= s^2 = 3/94$, $s = .1786$; α-Glu_C-1 $= p^2 + 2ps = 69/94$, $p^2 = .488$, and $p = .698$; and α-Glu_C-2 $= q^2 + 2qs = 5/94$, $q^2 = .0134$, and $q = .116$. α-Glu_C-3 $= r^2 + 2rs$ was not observed in the white population sample (but was seen in an Oriental group and in lymphoid-line cells). Since $p + q + r + s = 1$, then $r = .0068$. The estimated gene frequencies were α-$Glu_C^1 = .699$, α-$Glu_C^2 = .116$, α-$Glu_C^3 = .007$, and α-$Glu_C^0 = .179$. The maximum-likelihood estimates, calculated from a computer program, for the gene frequencies are: α-$Glu_C^1 = .703$; α-$Glu_C^2 = .107$; α-$Glu_C^3 = .016$; and α-$Glu_C^0 = .174$; from these frequencies for the four alleles, we then calculated the distribution of individuals of different phenotypes expected in the white population according to the hypothesis of four alleles in Hardy-Weinberg equilibrium (table 2). As opposed to the calculation assuming three alleles, the numbers of individuals observed did not differ significantly from the numbers expected under Hardy-Weinberg equilibrium for four alleles, including a null allele. Interestingly, approximately one-third of the population is heterozygous null. On the basis of the above gene frequencies, the observed numbers

TABLE 2

NO. INDIVIDUALS OF DIFFERENT α-GLU_C PHENOTYPES OBSERVED AND EXPECTED

	PHENOTYPE						
	1	2	3	2-1	3-1	3-2	0
No. observed	69	5	0	14	3	0	3
No. expected*	69.4	4.57	0.547	14.1	2.11	0.32	2.85

* Expected no. individuals in a sample of 94 were calculated on the basis of maximum-likelihood gene frequencies and four alleles in Hardy-Weinberg equilibrium.

of individuals determined from typing of long-term lymphoid-line cells also now fit that predicted by Hardy-Weinberg equilibrium. Although the number of black individuals studied was small, the relative phenotype frequencies appear to differ from those in other populations in that the α-Glu_C^2 allele appears to be more common.

Lymphoid-line cells or peripheral blood lymphocytes derived from members of four potentially informative families were examined. In all four (table 3), one parent was heterozygous 2-1 or 3-1 for neutral α-Glu_C and the other exhibited the common phenotype α-Glu_C-1. Offspring of two of the families (1 and 2) were α-Glu_C-1, and one child of an α-Glu_C-2-1 × 1 mating (family 3) was typed as a heterozygous α-Glu_C-2-1, all compatible with autosomal codominant inheritance. The fourth family, typed both from lymphoid-line cells and peripheral lymphocytes, was also of an α-Glu_C-1 × 2-1 mating, but their child typed as α-Glu_C-2 (all other polymorphic markers indicated that the child was a biological offspring of the parents). These results can be explained if the mother is genotypically α-Glu_C^{1-0}, the father α-Glu_C^{2-1}, and the daughter received the null allele from her mother and the 2 allele from the father, resulting in an α-Glu_C^{2-0} genotype and α-Glu_C-2 phenotype.

DISCUSSION

Genetic polymorphism is relatively common in several species with over 30 polymorphic enzymes and proteins having been described in man. From such studies, the average heterozygosity per locus in man has been estimated to be 7% [5]. Most protein polymorphisms have been detected by differences in charge or antigenic structure. However, there are two unusual features of the genetic polymorphism of neutral α-Glu_C reported here: the presence of a null allele, and the possible presence of an affinity allele.

There are only two previously described examples of genetic polymorphism detectable by differences in affinity rather than differences in charge. We previously described a genetic polymorphism for acid α-glucosidase detected by differences in affinity for the α-glucosidic linkages of the starch used as support for electrophoresis [1]. The serum cholinesterase polymorphism also appears to be detectable by affinity electrophoresis [6]. The increased anodal mobility of the α-Glu_C-3 isozyme compared with the common α-Glu_C-1 on starch, despite identical pIs, could similarly reflect decreased binding of the α-Glu_C-3 isozyme to the α-glucosidic linkages of the starch

TABLE 3

NEUTRAL α-GLU_C PHENOTYPES AND PROPOSED GENOTYPES OF FOUR FAMILY GROUPS

	PHENOTYPE			PROPOSED GENOTYPE		
FAMILY	Mother	Father	Child	Mother	Father	Child
1	3-1	1	1	3-1	1-1*	1-1*
2	1	2-1	1	1-1*	2-1	1-1*
3	1	2-1	2-1	1-1*	2-1	2-1
4	1	2-1	2	1-0	2-1	2-0

* Genotype could be 1-0.

matrix. However, the comigration of α-Glu_C-3 and α-Glu_C-1 in starch gel electrophoresis at pH 7.0 suggests that some other interaction might be operative [7]. Experiments similar to those previously utilized to define the affinity polymorphism for acid α-glucosidase would be required to determine if the α-Glu_C^3 represents a third affinity polymorphism [1]. The use of affinity electrophoresis could potentially reveal many new polymorphisms based not upon charge differences but upon differences in K_m or binding sites of proteins for various substrates, inhibitors, and cofactors.

We found a null allele for human neutral α-Glu_C which has a gene frequency of .174. This represents one of the highest gene frequencies for a null allele in an outbred population. Only the O blood group at the *ABO* locus (presumably representing a null for a glycosyl transferase) (European population = .685) [8] and E_2 (C_5^-) locus for serum cholinesterase (.95 gene frequency) [9] represent loci with a null allele with substantial gene frequency in an outbred population, and which do not apparently cause disease in the homozygote.

Null alleles have been detected in the past from studies of metabolic diseases, of inheritance of polymorphic markers in families, and of populations. Null alleles which result in disease are usually ascertained despite the fact that the gene frequencies for these null alleles are low and the disease state is rare. Occasionally, in certain populations, the null allele for such disorders occurs with polymorphic frequencies. Thus, α and β thalassemia in the Mediterranean and in Middle and Far Eastern countries [10, 11] and Tay-Sachs disease in Ashkenazi-Jewish populations [12] have a particularly high incidence. Anomalous inheritance of polymorphic markers within families has also resulted in detection of null alleles. In most cases, such families have been ascertained because the homozygous null state results in disease. Thus, as two of many examples, anomalous inheritance of the relevant polymorphic marker has been shown in families of patients with deficiency of adenosine deaminase [13] and α-fucosidase [14]. Null alleles not known to result in disease have also been ascertained by family studies of the inheritance of polymorphic markers [5]. In theory, an estimate of the frequency for such alleles could be obtained by comparing observed with expected mother-child phenotypes [15]. In most of the examples of null alleles detected only by anomalous inheritance in families, the phenotype of the homozygous deficient is unknown. However, three apparently healthy individuals who were homozygous null for LDH-B [16], 6PGD [17], and PGM_1 [18], respectively, have been found during population surveys. The locus for haptoglobin may also exhibit a null allele, but since there are other nongenetic causes of ahaptoglobinemia, the apparently homozygous null for haptoglobin could be due to several different disease states [15].

There have also been several reported instances of a null or silent allele with high gene frequencies which do not give rise to disease, but they have been found only in small, isolated population groups. In the Alaskan Eskimo population, the "null" gene for serum cholinesterase was found to have a gene frequency of .12 [19]. Later, Scott showed that the null gene frequency actually consisted of two alleles for the lack of enzyme activity: one for total lack of activity, and the other for partial activity, together giving an apparent gene frequency of .12 [20]. In South West Africa, an unstable enzyme of red cell adenosine deaminase is found in 2%–3% of the Kung Bushman

population [21]. Again, in South West Africa, one individual was found to be deficient for red cell esterase D in a Kwambi population with a gene frequency of .12 [22]. In the African Pigmy population, a null gene for red cell peptidase C was found to have a gene frequency of .20 [23]. Once the null allele has been established in these groups, inbreeding maintains the gene frequency at polymorphic levels.

Detection of a null allele which does not result in disease in the homozygous state raises special problems since a null allele can only be recognized in population studies by virtue of the asymptomatic rarer homozygous state. Thus, it would require a sample size of 45,000 to detect with 99% probability a null allele which occurs with a gene frequency of .01, compared to a sample size of approximately 230 to detect an electrophoretic variant with the same gene frequency; conversely, a gene frequency of .14 would be required to detect a null allele with the same degree of certainty as an electrophoretic variant with a gene frequency of .01 (the arbitrary definition of a polymorphism with two alleles). In an attempt to estimate the relative frequency of null alleles compared to that of other polymorphic alleles, we reviewed the literature to determine the number of polymorphic loci in which one of the rarer alleles occurs with a gene frequency of .14 or greater. Fifty percent (18 of 36) polymorphic loci contain a rarer allele with a gene frequency of .14 or greater in one or more of the populations studied [24]. This observation suggests that null alleles, independent of the difficulties of ascertainment, occur to a lesser extent than other types of genetic polymorphisms. It is likely that both a smaller portion of the genome is at risk for a "null" mutation (i.e., only the portion of the molecule involved in the catalytic or binding site), and that the genetic mechanisms which give rise to a null allele are more limited than those which lead to change in charge. These factors could account for the lower frequency of null alleles. Alternatively, null alleles would not be expected to be neutral and, therefore, except for new mutations, should have been eliminated during evolution. Newer techniques of DNA hybridization, potentially, will provide better estimates of the incidence of null alleles [25].

"Null" phenotypes could encompass a variety of mutations. Most of the null alleles for enzymes have been detected in red cells in contrast to the null allele for neutral α-Glu_C. In a long-lived, nonprotein synthesizing cell, such as a red cell, an apparent null allele could easily be due to an unstable mutant enzyme. In some cases (6PGD, ADA, and PEPC) where white blood cells have been examined, enzyme activity has indeed been detectable [17, 21, 26]. True null alleles could represent mutations ranging from total deletion of the gene to the presence of a protein with altered activity or affinity only for the particular substrate used for testing. For example, a rare mutant enzyme of hexosaminidase A displays no activity against the artificial substrate but retains normal activity against the natural substrate [27]. The opposite case has also been described [28]. Therefore, the apparent null allele for α-Glu_C could represent enzyme with altered substrate specificity, that is, null for the low molecular weight artificial substrate while retaining activity upon a high molecular weight natural substrate. However, at least one of these null individuals has no detectable activity on the natural substrate glycogen ($< 5\%$) as well as no detectable activity on the artificial substrate. It remains to be determined if these individuals have trace activity for the high molecular weight natural substrate, which could explain why the three homozy-

gous null individuals for α-Glu_C are apparently healthy. Alternatively, these individuals may have increased susceptibility to as yet unidentified disease(s) as do group O individuals for peptic ulcer [29] and those with complete α_1-antitrypsin deficiency for emphysema [30]. Surveys of larger populations would be required to detect such effects.

Finally, the coincident occurrence of the neutral α-Glu_C null allele and the various different deficiency alleles at the acid α-glucosidase locus could affect the phenotypic expression of complete or partial acid α-glucosidase deficiency. Such interaction could explain the recently reported simultaneous occurrence of infantile and adult onset acid α-glucosidase deficiency in the same family [31].

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