Hum Genet (1987) 77:241–245

Human Genetics

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Functional hemizygosity in the human genome: direct estimate from twelve erythrocyte enzyme loci

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Summary. Cord blood samples from 2020 unrelated newborns were screened for levels of enzyme activity for twelve enzymes. The level of enzymatic activity for 100 determinations were consistent with the existence of an enzyme-deficiency allele. The frequency of deficiency alleles in the Black population (0.0071) was four times higher (after removal of the G6PD*A$^-$ variant) than in the Caucasian sample (0.0016). These frequencies are approximately double the frequency of rare electrophoretic mobility variants at similar loci in the same population. Given the number of functionally important loci in the human genome, these enzyme deficiency variants could constitute a significant health burden.

Introduction

Increasing evidence is accumulating that the genome of each individual may be functionally hemizygous at from several to many autosomal loci, in addition to the loci located on the sex chromosome. That is, one member of the allelic pair at an autosomal locus is either absent (molecular hemizygosity) or is unable to direct the synthesis of functional gene product (biochemical hemizygosity). The level of hemizygosity in the human genome has a direct impact on the frequency of both genetic disease and reproductive failure (Carter 1977, 1982; Neel 1979; Vogel 1984; Costa et al. 1985). Hemizygosity also has a direct effect on the estimated health cost of mutational damage in that a functionally significant mutation at a hemizygous locus would appear to be inherited as a dominant trait and would be of significance in the first generation (Mohrenweiser 1983; Searle and Edwards 1986).

Examples of hemizygosity that have received recent attention are the several heritable tumors that are associated with a predisposing event involving the apparent absence of genetic material (Cavenee et al. 1986; Seizinger et al. 1986). That is, the individual is apparently hemizygous at a locus associated with tumor development and therefore a single somatic event is sufficient to initiate tumorogenesis. Molecular hemizygosity in the genome, identified with Southern blotting techniques and not associated with disease conditions, has also been identified. These known hemizygous segments include regions adjacent to several defined genes (Bell et al. 1980; Rotwein et al. 1983), variation in the number of copies of genes (Erickson and Schmickel 1985), and variation in the copy number of repetitive elements (Jeffrey et al. 1985). Molecular studies of a series of genetic diseases outline the range of events, considered as "point mutations", which at the physiological/metabolic level of cellular function appear to be null alleles. These events range from the complete or partial deletion of a gene (Monaco et al. 1986; Rozen et al. 1985; Spritz and Forget 1983; Yang et al. 1984) to seemingly innocuous base substitutions (Busslinger et al. 1981; Daar et al. 1986; Spritz et al. 1981). Although the frequency of hemizygosity at any single locus associated with a genetic disease or a genetic predisposition to a disease appears to be small, it would appear that the total hemizygosity in the genome may be significant when summed across the estimated 50–100,000 expressed loci of the human genome (Office of Technology Assessment 1986).

Within the context of the development of a program to study the germinal mutation rate in human populations (Neel et al. 1987), we have now examined erythrocyte samples from 2020 unrelated newborns, including those from previous reports (Mohrenweiser 1981, 1983), for the presence of a level of enzyme activity consistent with the existence of an enzyme deficiency allele at twelve erythrocyte enzyme loci. Data from similar studies in other populations will be reviewed in order to estimate the apparent level of detectable hemizygosity as manifested by a specific reduction in enzyme activity for a set of loci encoding enzyme proteins. The data will be contrasted with the frequency of rare electrophoretic mobility variants in the same populations.

Materials and methods

Umbilical cord blood samples were obtained from newborns at the University of Michigan Women's Hospital. Informed consent and blood samples were obtained from parents; the samples were stored in liquid nitrogen (Neel et al. 1979). Adenylate kinase (AK, EC 2.7.4.3); aspartate aminotransferase (GOTS, EC 2.3.1.1); enolase (ENO, EC 1.2.1.11); phosphoglyceromutase (DPGM, EC 2.7.5.3); glyceraldehyde-3-phosphate dehydrogenase (GAPD, EC 1.2.1.12); glucose-phosphate isomerase (GPI, EC 5.3.1.9); glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49); lactate dehydrogenase (LDH-B, EC 1.1.1.27); malate dehydrogenase (MDH, EC 1.1.1.37); phosphoglycerate kinase (PGK, EC 2.7.2.3); pyruvate kinase (PK, EC 2.7.1.40); and triosephosphate isomerase (TPI, EC 5.3.1.1) assays were performed as described by Fielek and Mohrenweiser (1979), Mohrenweiser (1983), and Mohrenweiser and Fielek (1982). The stability characteristics of each enzyme as well as the influence of reticulocyte per-

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Table 1. Distribution and frequency of erythrocyte-enzyme-deficiency variants among loci in unselected newborns

Enzyme	Number of occurrences										Total[b] variants/ determination
	Caucasian					Black					
	Normal activity (%)					Normal activity (%)					
	< 50 (< 10)[a]	50–55 (10–15)	56–60 (16–20)	61–65 (21–25)	Variants/ determination	< 50 (< 10)[a]	50–55 (10–15)	56–60 (16–20)	61–65 (21–25)	Variants/ determination	
AK	0	0	0	2	2/1714	0	0	0	0	0/168	3/1988
DPGM	0	1	0	1	2/1087	0	0	0	0	0/ 85	3/1236
ENO	2	3	0	1	6/1094	1	0	0	0	1/ 85	10/1246
GAPDH	0	0	0	0	0/1035	0	0	0	0	0/ 84	0/1183
GOTS	0	0	1	1	2/1592	0	0	0	1	1/161	3/1854
GPI	2	1	0	1	4/1712	0	0	0	0	0/168	4/1986
LDH	0	0	0	0	0/1736	0	0	0	0	0/170	0/2013
MDH	0	1	0	0	1/1736	0	0	0	1	1/170	2/2013
PGK	0	0	0	0	0/1736	0	0	0	0	0/170	0/2013
PK	1	4	2	11	18/1736	0	0	0	4	4/170	23/2013
TPI	2	1	3	3	9/1713	1	0	1	5	7/168	17/1987
G6PD (Female)	6	3	1	0	10/ 821	4	2	0	2	8/ 71	19/ 952
G6PD (Male)	0	0	2	1	3/ 908	9	3	0	0	12/ 97	16/1052
G6PD (Total)					13[c]/1729					20[d]/168	35[c,d]/2004
Total					57/18,620					34/1767	100/21,536

[a] Activity for sex linked trait
[b] Total includes about 100 probands that could not be assigned to the other two ethnic groups
[c] Includes one mobility variant, G6PD*CAA1
[d] Includes 11 variants with G6PD*A^- mobility

centage and mean cell age on the level of enzyme activity in both adult and cord blood samples have been described (Mohrenweiser et al. 1981). Only the first-born child in any family has been included in the current sample, in contrast to the report on mutation rates in this population by Neel et al. (1987) where, with the exception of identical twins, all newborns were included. Approximately 3%–4% of the probands were considered premature (birth weight < 2000 g) or exhibited a congenital defect (Neel and Mohrenweiser 1984).

Results

Functional null or enzyme-deficiency variants were defined by a level of enzymatic activity that was less than 65% of the normal or control mean activity and concurrently, a value at least 3 standard deviations *(SD)* below the population mean (Mohrenweiser 1981). The latter constraint has proven to be redundant in that the coefficient of variation among individuals within the newborn or the adult population is less than 12% for each of the twelve enzymes included in the study and therefore 65% of control is always more than three SDs from the mean (Mohrenweiser 1983). In addition, for the reduced activity to be classified as the result of an enzyme-deficiency allele, the activity of other enzymes assayed in this individual sample must be within the normal range. Thus, abnormal results associated with generalized hemoglobinopathies or other traits not involving a specific enzyme locus would be excluded from further consideration. This requirement of a normal level of activity for other erythrocyte enzymes also serves as a control for sample quality. Only a single example of a proband with two deficiency alleles was observed. This male child inherited the TPI deficiency allele from the father and the G6PDA^- allele from his mother.

The results from screening samples from 1740 Caucasian and 172 Black unrelated newborns are shown in Table 1. The total group includes additional individuals from other races (1%) as well as individuals of mixed or unknown racial origins (4%). In 100 of the 21,536 determinations, the level of enzymatic activity was consistent with the existence of an enzyme-deficiency variant. A genetic basis for the reduced activity was confirmed for each variant by the detection of a reduced level of enzyme activity in erythrocytes from one of the parents. Fifty-seven of the variants were identified among 18,620 determinations (3.1 variants per 1000 determinations) from Caucasian newborns while 34 variants were detected among 1767 determinations (19.2 variants per 1000 determinations) from Black newborns.

Enzyme-deficiency variants were detected in this study at nine of the twelve loci screened. Deficiencies at the other three loci have been identified in other studies and have been reported in the clinical literature. An LDHB null variant has been identified as a polymorphism in a Central American Indian population (Tanis et al. 1977; Mohrenweiser and Novotny 1982); while deficiencies of PGK and GAPDH as well as LDH have been reported, although they are less frequent than many of the other enzymopathies (Beutler 1978; Kahn et al. 1979). Thus, it is probable that the absence of deficiency variants at these three loci, in this study, reflects the frequencies of the deficiency alleles in the population studied in Ann Arbor rather than a failure of the analytical procedures.

The range of residual activity in the probands (with the exception of males with G6PD deficiency) ranged from 44%–64% of normal and from 3.3 to 5.4 *SD*s below the mean. In all

families, one parent had a level of enzyme activity consistent with the profile observed in the proband. The affected parents were evenly distributed between mothers and fathers. Thus, the marked reduction in enzyme activity had characteristics of a recessively (or sex-linked, in the case of G6PD deficiency) inherited genetic trait. The specificity of the reduction, that is, restriction to a specific enzyme, and codominant inheritance would be consistent with the existence of a molecular lesion involving the structural gene or the immediate flanking regions for the enzyme exhibiting the reduced activity. In general, genetic variation that does not involve the structural locus would not usually be inherited as a codominant trait, as are the nonsex-linked traits observed in this study.

The frequency of enzyme-deficiency alleles in the Caucasian sample is 0.00161 while the frequency in the Black sample is about six times higher, namely 0.01010. The relatively high frequency of G6PD deficiencies in Blacks and PK deficiencies in Caucasians is consistent with the clinical data on enzymopathies and the frequency of the G6PG*A$^-$ variant in the Black population (Beutler 1979; Motulsky and Yoshida 1969). Of the 20 G6PD deficiencies in Blacks, 11 were associated with the electrophoretically detectable A$^-$ allele. Excluding these 11 variants from the deficiency class reduces the frequency of such variants to 0.0071 in the Black group, a frequency still more than four times the frequency in the Caucasian group. One of the G6PD deficiencies in the Caucasian group was associated with a variant with C-like electrophoretic mobility. With the exception of the above mentioned G6PD mobility variants, none of the other deficiency variants were associated with an identifiable alteration in electrophoretic mobility. The existence of a weakly staining mobility variant would be consistent with the deficiency being associated with an unstable enzyme molecule, as is known for the G6PD*A$^-$ variant. The absence of detectable mobility variation does not exclude an unstable enzyme molecule as the cause of the deficiency. The interchanged amino acids may have had similar charges or the residual enzyme activity may have been too low to be detected following electrophoresis, especially in the heterozygote individual. Failure to produce a recognizable gene product or the existence of an inactive enzyme subunit are other possible explanations for the absence of detectable functional gene product.

As previously noted in preliminary reports from this newborn screen, the high frequency of the TPI-deficiency allele, especially among Blacks, was surprising (Mohrenweiser 1983; Mohrenweiser and Fielek 1982). Reports of homozygosity for TPI deficiency in the clinical literature are relatively rare with only about thirty reported cases in the world including only four individuals in three families in the U.S. (Maquat et al. 1985; Mohrenweiser and Fielek 1982; Rosa et al. 1985). This contrasts with the expected frequency of at least 1 in 2000 Black newborns, given that the deficiency allele frequency is 0.023 in this population. No homozygous deficient probands were observed in this study, as expected given the number of Black newborns screened. Because of the severity of the symptoms, the homozygous deficient individuals should be observed in clinical studies if they survive to birth. Studies of TPI-deficient mice indicate that homozygosity for several different TPI null alleles is apparently an embryo lethal condition, in that no homozygous deficient animals are observed (Charles and Pretsch 1987). Given the apparent lethality of the homozygous condition and the calculation that TPI is the least rate-limiting step in glycolysis (Albery and Knowles 1976), it is difficult to identify a mechanism for the maintenance of this allele frequency.

A direct comparison of the relative frequency of deficiency and mobility variants can be made at nine loci (excluding DPGM, PK, and GAPD, which were not studied for mobility variants). In the Caucasian sample, the frequency of alleles for enzyme deficiencies at these nine loci was 0.00169 compared to an allele frequency of 0.00083 (35 variants/42,111 alleles) for rare mobility variants at the same loci (Mohrenweiser et al. 1987). Similar figures in the Black sample were 0.0078 for deficiency alleles (excluding G6PD*A$^-$) and 0.00088 (3 variants/3395 alleles) for rare electrophoretic mobility alleles (G6PD*A is a polymorphic variant and therefore is excluded as a rare variant in the Black sample). Data are available on the frequency of alleles for rare electrophoretic mobility variants at forty erythrocyte enzyme loci in these populations, the frequencies being 0.00056 for Caucasians and 0.00159 for Blacks. Thus, the frequency of deficiency variants was 100%–250% greater than the frequency of rare electrophoretic mobility variants, irrespective of the data base of electrophoretic mobility variants used for the comparison.

Discussion

Three other studies of human populations have employed similar techniques to screen for enzyme-deficiency alleles. Eber et al. (1984) and Krietsch et al. (1977) studying only 5 erythrocyte enzymes, reported seven variants in about 3000 individuals (0.5 variants/1000 determinations) in Germany. The null-allele frequency (recalculated from their data using 65% of normal as the definition for a deficiency variant) is 0.00028. They also identified additional individuals with lesser degrees of activity reduction, which are not included in the above frequency calculation but which had characteristics consistent with a heritable genetic trait. Satoh et al. (1983) in Japan, studying 11 enzymes, found 61 enzyme-deficiency variants in 26,634 determinations (2.3 variants/1000 determinations) for an enzyme-deficiency-allele frequency of 0.00131. Mohrenweiser and Neel (1984) detected 11 enzyme-deficiency variants in 6741 determinations (1.6 variants/1000 determinations) among samples from several Amerindian tribes. Combining these data with the two data bases from this study, one obtains an overall unweighted mean frequency of 0.00396 for enzyme-deficiency alleles for the five populations.

The apparent differences among ethnic or geographical groups in the frequency of enzyme-deficiency variants reflect, at least in part, differences in the battery of loci studied in the different populations (low figure for Germany), but they also suggest possible ethnic differences (high value for U.S. Blacks). The frequencies observed in the study in Germany are similar to the frequencies at the same loci in the other populations, with the exception of the TPI deficiency in Blacks. The higher frequency of deficiency variants among Blacks is similar to a higher frequency of mobility variants observed in this same population (Neel and Mohrenweiser 1987; Mohrenweiser et al., in preparation). The distribution of deficiency variants (excluding G6PDA$^-$) among loci is similar in the Black and Caucasian groups with G6PD, TPI, and PK accounting for 70% and 60% of the variants. The estimates of the frequency of enzyme-deficiency variants are not inconsis-

tent with estimated frequencies obtained for other loci from other studies (Martin 1983; Gershowitz 1983).

Similar null-variant frequencies have also been observed in other species. Voelker et al. (1980a) and Langley et al. (1981) have reported frequencies of 2.6 and 2.3 null variants/1000 determinations in two natural populations of *Drosophila melanogaster*. Bulfield et al. (1984) identified 3.69 low-activity variants/1000 determinations in screening wild-caught mice *(Mus musculus)*.

Results from several experiments suggest that de novo mutations associated with enzyme deficiency are at least as frequent as mobility mutants. In a study of the spontaneous mutation rate in *Drosophila,* null mutants occurred at a rate at least three times the rate for mobility mutants (Mukai and Cockerham 1977; Voelker et al. 1980b). Null mutants have been detected with a frequency similar to that of electrophoretic mobility mutants in the offspring of mice exposed to chemical mutagens (Lewis and Johnson 1983, 1986; Charles and Pretsch 1987). The exception to this generalization, of an approximately equal frequency of null and mobility mutants, is the results from two radiation mutagenesis studies in mice, where all seven of the mutations were nulls (Malling and Valcovic 1977; Charles and Pretsch 1986). Racine et al. (1980) also reported an increased null-mutation frequency in the progeny of irradiated *Drosophila.*

The biochemical basis for the deficiency variants will generally be either (a) the absence of a protein, (b) the presence of a nonfunctional enzyme, or (c) the altered stability of the enzyme. As the erythrocyte is unable to synthesize protein, the reduced stability of an enzyme molecule is a significant cause of erythrocyte enzymopathies although it does not often cause a significant metabolic block in nucleated cells.

The molecular basis for the null variants can range from deletion of the entire allele through single base deletions, which could result in removal of important regulatory sequences and mRNA processing sites to frameshifts. They could also result from point mutations that alter regulatory sequences or processing sites or result in amino acid substitutions causing enzyme instability, inappropriate processing, or the synthesis of a nonfunctional enzyme molecule (Mohrenweiser 1983). This spectrum of events that can give rise to enzyme-deficiency variants contrasts with the situation for mobility variants, which are almost exclusively associated with nucleotide substitutions in exons, which therefore give rise to the interchange of amino acids with nonidentical charge.

Assuming that this estimate of the enzyme-deficiency allele frequency is typical of the average for the remainder of the human genome, one can begin to estimate the total level of hemizygosity. With a deficiency-allele frequency of 0.00396, the frequency of homozygosity at any locus is 1.56×10^{-5}. Estimates of the number of loci in the human genome generally range from 50 to 100,000. Thus, each individual could be hemizygous at 200–400 loci in addition to loci located on the X chromosome. Assuming that 5000 of these autosomal loci are critical for codominantly inherited traits, such that the absence of a functional gene product is associated with disease, then each conceptuses would be hemizygous at 20 critical loci. With these same assumptions, 7% of conceptions would be homozygous at a critical locus. It is possible that 5%–10% of the spontaneous abortions [estimated at 50% of conceptions (Edmonds et al. 1982; Miller et al. 1980) and 30% of the recognized spontaneous abortions being due to detectable chromosomal anomalies] could be associated with homozygosity for functionally significant enzyme-deficiency variants. This fetal loss plus the diseases associated with single-gene defects constitute a significant health burden for the human population (Neel 1979). Additionally, this background level of hemizygosity increases the potential health burden of new mutations. A portion of the de novo null mutations will exhibit characteristics of a dominant trait since a single event at a locus that is constitutively hemizygous will be manifested in the first generation (Mohrenweiser 1983; Searle and Edwards 1986). This contrasts with the usual expectation that "point" or "gene" mutations will only have heterozygous and limited genetic health consequences for many generations (Denniston 1982; Carter 1982).

Acknowledgement. This work was supported by Department of Energy contract DE-AC02-82ER-60089. The technical assistance and helpful discussions of many individuals are gratefully acknowledged, especially the assistance of S. Fielek and the discussions with J. V. Neel.

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Received February 18, 1987 / Revised May 18, 1987