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Gaucher Disease: Gene Frequencies in the Ashkenazi Jewish Population

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Summary

DNA from over 2,000 Ashkenazi Jewish subjects has been examined for the four most common Jewish Gaucher disease mutations, which collectively account for about 96% of the disease-producing alleles in Jewish patients. This population survey has made possible the estimation of gene frequencies for these alleles. Eighty-seven of 1,528 individuals were heterozygous for the 1226G (N370S) mutation, and four presumably well persons were homozygous for this mutation. The gene frequency for the 1226G allele was calculated to be .0311, and when these data were pooled with those obtained previously from another 593 Jewish subjects, a gene frequency of .032 with a standard error of .004 was found. Among 2,305 normal subjects, 10 were found to be heterozygous for the 84GG allele, giving a gene frequency of .00217 with a standard error of .00096. No examples of the IVS2(+1) mutation were found among 1,256 samples screened, and no 1448C (L444P) mutations were found among 1,528 samples examined. Examination of the distribution of Gaucher disease gene frequencies in the general population shows that the ratio of 1226G mutations to 84GG mutations is higher than that in the patient population. This is presumed to be due to the fact that homozygotes for the 1226G mutation often have late-onset disease or no significant clinical manifestations at all. To bring the gene frequency in the patient population into conformity with the gene frequency in the general population, nearly two-thirds of persons with a Gaucher disease genotype would be missing from the patient population, presumably because their clinical manifestations were very mild.

Introduction

Gaucher disease is an autosomal recessive disorder that is most prevalent in the Ashkenazi Jewish population. Variable expressivity of the homozygous form of an autosomal recessive disorder makes it virtually impossible to arrive at accurate estimates of gene frequencies from the disease incidence. When accurate detection of heterozygotes is technically impossible, then this route to calculation of gene frequencies is also closed. This, until recently, has been the state of affairs with respect to the frequency of Gaucher disease. On the basis of the occurrence of homozygotes in the Ashkenazi Jewish population, Fried estimated a disease incidence of 1:7,750 (Fried 1958) and 1:10,000 (Fried 1973). In contrast, estimates based on the enzymatic activities of leukocytes led to predicted disease-incidence figures of 1:640 (Kolodny et al. 1982), 1:2,003 (Grabowski et al. 1982), and 1:3,969 (Matoth et al. 1987).

We have recently shown that four Gaucher disease mutations account for about 96% of the disease-producing mutations in Jewish patients (Beutler et al. 1992). Facile PCR-based screening techniques have now been applied to DNA samples from over 2,000 Ashkenazi Jewish subjects, allowing us to make relatively accurate estimates of the gene frequencies in this population for the first time.

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Subjects, Material, and Methods

Subjects

Samples were obtained from presumably normal persons of Ashkenazi Jewish ancestry from a variety

of sources, but the great majority of samples came from adolescents being screened for Tay-Sachs disease in the program of the Committee for Prevention of Jewish Genetic Diseases of Dor Yeshorim. The geographical origin of the ancestors of these high school students was broadly distributed throughout Russia, Poland, Lithuania, and other countries of eastern Europe and is not notably different from that of other Ashkenazi Jews. There is a moderate amount of inbreeding in this population; some of the subjects are from small, tight-knit religious communities in which cousin marriages, although discouraged, are permitted. Criteria for diagnosis of the Jewish patients with Gaucher disease have been described elsewhere (Beutler 1992; Beutler et al. 1992).

DNA Analysis

DNA was extracted from whole blood either by using an Applied Biosystems (Foster City, CA) DNA extractor or by a modification of the rapid method described by Innis et al. (1990). Briefly, 50 μl of whole blood was pipetted into a 1.5-ml Eppendorf tube. After the addition of 500 μl of 10 mM Tris-HCl, 1 mM EDTA pH 7.5 (TE), the tube was spun in a microcentrifuge at 13,000 *g* for 30 s. The supernatant was decanted, and the pellet was resuspended in 500 μl TE, mixed, and centrifuged for 30 s. This step was repeated for a total of four washes with TE, to remove the hemoglobin. After the final washing the pellet was resuspended in 100 μl of PCR buffer consisting of 33.5 mM Tris-HCl pH 8.8, 8.3 mM $(NH_4)_2SO_4$, 3.35 mM $MgCl_2$ containing 0.5% (v/v) Tween 20, and 100 μg of proteinase K/ml. The suspension was incubated for 1 h at 55°C and then was heated at 95°C for 10 min to inactivate the proteinase K. Five microliters of this DNA preparation was used in a 60-μl PCR system.

Samples were examined for mutations 84GG, IVS2(+1), 1226G (N370S), and 1448C (L444P), by allele-specific oligonucleotide hybridization (ASOH) after amplification of two segments of the genomic DNA by simultaneously using two pairs of oligonucleotide primers, as shown in table 1. For technical and logistic reasons, not all of the samples were examined for all of the mutations.

Results

The results of our studies are summarized in table 2. Included in the table are the results we reported elsewhere on 593 samples examined only for the 1226G mutation (Zimran et al. 1991).

Discussion

The study of a large number of Ashkenazi Jewish subjects has made it possible for us to establish more accurately the gene frequencies for Gaucher disease-producing genes in this population. Among the 1,528 subjects examined for the 1226G mutation in the present investigation, 87 heterozygotes and 4 homozygotes were found. The combined gene frequency for the 1226G allele from both the present study and our earlier study (Zimran et al. 1991) is .032 with a standard error of .004. Of 2,305 normal subjects, 10 were found to be heterozygous for the 84GG allele, giving a gene frequency of .00217 with a standard error of .00096. No instances of the IVS2(+1) mutation were found among 1,256 samples screened, and no 1448C mutations were found among 1,528 samples examined.

The fact that some homozygotes for the 1226G mutation were found in the normal population is not surprising, especially when young subjects are being

Table 1

PCR Primers and ASOH Probes Used in Screening for Four Common Gaucher Disease Mutations

Primer	Probes	Sequence Detected
979T 5′-GAATGTCCCAAGCCTTTGA-3′	5′-ACAGGATTGCTTCTACT-3′	84G Normal
1336B 3′-GCTAAGAGAACGAAGTCGAA-5′	5′-ACAGGATTGGCTTCTACT-3′	84GG Mutant
	5′-GGCATCAGGTGAGTGAG-3′	IVS2 Normal
	5′-GGCATCAGATGAGTGAG-3′	IVS2 Mutant
5183T 5′-CAAGGTCCAGGATCAGTTGC-3′	5′-TACCCTAGAACCTCCTG-3′	1226 Normal
653B 3′-TCACCCGACTTCTGTCGCAA-5′	5′-TACCCTAGAGCCTCCTG-3′	1226 Mutant
	5′-GAACGACCTGGACGCAG-3′	1448 Normal
	5′-GAACGACCCGGACGCAG-3′	1448 Mutant

Table 2

Frequency of Four Gaucher Disease Mutations among Over 2,000 Normal Ashkenazi Jewish Subjects

Mutation	No. of Samples	No. of Normal Samples	No. of Heterozygous Samples	No. of Homozygous Samples	Gene Frequency
1226G:					
Present study	1,528	1,437	87	4	.0311 } .0321
Zimran et al. 1991	593	554	37	2	.0346 }
84GG	2,305	2,295	10	0	.00217
IVS2(+1)	1,256	1,256	0	0	
1448C	1,528	1,528	0	0	

screened. The median age at onset of symptoms or age at diagnosis, whichever was first, is about 30 years in persons with this genotype (Beutler 1991, 1992), and, in one-fourth of the patients, no symptoms are present before the age of 45 years (Beutler 1992). A similar observation was made in our previous study (Zimran et al. 1991) where two homozygotes for this mutation were encountered, one of whom was totally without stigmata of Gaucher disease. The other homozygous subject has been found since the earlier survey was published, and she has mild manifestations of the disease (G. A. Grabowski, personal communication).

Our studies also reveal a discrepancy between the ratio of 1226G alleles to 84GG alleles in the general population and in the patient population. If all individuals with two Gaucher disease–causing alleles were represented in the patient population, then the proportion of the different Gaucher disease–producing alleles would be the same in the patient population as it is in the general population. Table 3 presents an update of our studies of the prevalence of different Gaucher disease genotypes in Ashkenazi Jewish patients (Beutler 1992; Beutler et al. 1992), now comprising 121 patients. The ratio of the 1226G mutation to the 84GG mutation is 185:29 (6.38:1). However, in the general Ashkenazi Jewish population we find that the relative frequency of the 1226G mutation is 14.8 times as great as that of the 84GG mutation. Such a distortion in the frequency of alleles in the patient population would occur if some of the gene combinations were lethal or if some produced no clinical symptoms or produced clinical symptoms so mild that the affected individuals did not present as patients. Some combinations probably are lethal, since they have never been observed. A salient example is homozygosity for the 84GG frameshift mutation. However, since the latter genotype would be predicted to occur only once in 212,521 births in the Jewish population it could not materially affect gene frequencies. Given the fact that disease expression in the 1226G/1226G homozygote is often very mild and that onset is late in life, the most likely reason for the discrepancy between the incidence of these two most-common mutations in the patient and in the general population is that many patients with this genotype are never diagnosed. We

Table 3

The Observed and Expected Distribution of Gaucher Disease Genotypes in the Ashkenazi Jewish Population

Genotype	Observed No.	Expected No.
1226G/1266G	65	187
1226G/84GG	28	28
1226G/1448C	9	9
1226G/IVS2(+1)	6	6
1226G/Other	12	12
84GG/Other	1	1
Total	121	243
Allele		
1226G allele	185	429
84GG allele	29	29
1226G:84GG	6.38	14.8

NOTE. — The observed genotypes are based on 121 unrelated Jewish patients, updated from a previous publication (Beutler 1992). The expected distribution of genotypes shown is provided by supplementing the patient population with sufficient additional individuals homozygous for the 1226G/1226G genotype to provide the same ratio of 1226G:84GG mutations (14.8:1) as found in the general population. The number of patients with other genotypes has been held constant. This analysis implies that over one-half of individuals with a Gaucher disease genotype have insufficient clinical manifestations to have been diagnosed and to present as patients.

already realized, from earlier studies and from the fact that asymptomatic homozygotes for this mutation were found in the present survey, that such homozygotes may be free of clinical symptoms and therefore might not seek medical attention. The absence of these individuals from the patient cohort accounts for the fact that the relative percentage of 84GG alleles among the patients is exaggerated.

To restore the ratio of these two alleles in the patient population to that found in the general population, 122 additional 1226G/1226G homozygotes would need to be included in the Gaucher disease patient population. The actual and expected distribution of genotypes in the patient population is summarized in table 3. Any estimate of clinically inapparent homozygotes for the 1226G mutation will be imprecise because of the high standard error of the estimate of the gene frequency of the 84GG allele, but, on the basis of the data now available, the best estimate we can make is that only one-third of the 1226G/1226G homozygotes are patients with Gaucher disease.

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References

Beutler E (1991) Gaucher's disease. N Engl J Med 325: 1354–1360

——— (1992) Gaucher disease: new molecular approaches to diagnosis and treatment. Science 256:794–799

Beutler E, Gelbart T, Kuhl W, Zimran A, West C (1992) Mutations in Jewish patients with Gaucher disease. Blood 79:1662–1666

Fried K (1958) Gaucher's disease among the Jews of Israel. Bull Res Counc Isr Proc Fourth Meet Isr Genet Circle 7B: 213

——— (1973) Population study of chronic Gaucher's disease. Isr J Med Sci 9:1396–1398

Grabowski GA, Dinur T, Gatt S, Desnick RJ (1982) Gaucher type 1 (Ashkenazi) disease: considerations for heterozygote detection and prenatal diagnosis. In: Desnick RJ, Gatt S, Grabowski GA (eds) Gaucher disease: a century of delineation and research. Alan R Liss, New York, pp 573–595

Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) (1990) PCR protocols: a guide to methods and applications. Academic Press, San Diego

Kolodny EH, Ullman MD, Mankin HJ, Raghavan SS, Topol J, Sullivan JL (1982) Phenotypic manifestations of Gaucher disease: clinical features in 48 biochemically verified type I patients and comment on type II patients. In: Desnick RJ, Gatt S, Grabowski GA (eds) Gaucher disease: a century of delineation and research, Alan R Liss, New York, pp 33–65

Matoth Y, Chazan S, Cnaan A, Gelernter I, Klibansky C (1987) Frequency of carriers of chronic (type I) Gaucher disease in Ashkenazi Jews. Am J Med Genet 27:561–565

Zimran A, Gelbart T, Westwood B, Grabowski GA, Beutler E (1991) High frequency of the Gaucher disease mutation at nucleotide 1226 among Ashkenazi Jews. Am J Hum Genet 49:855–859