Am. J. Hum. Genet. 49:855–859, 1991

High Frequency of the Gaucher Disease Mutation at Nucleotide 1226 among Ashkenazi Jews

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Summary

Reliable estimates of the frequency of Gaucher disease–producing mutations are not available. The high frequency of Gaucher disease in the Ashkenazi Jewish population is due to the occurrence of a mutation at nucleotide (nt) 1226. We have screened 593 DNA samples from normal Ashkenazi Jews, as well as 62 DNA samples from all of our Ashkenazi Jewish patients with Gaucher disease, for the presence of the 1226 mutation. In the 593 presumed normal Ashkenazi Jewish individuals the 1226 mutation was identified in the heterozygous state in 37 and in the homozygous state in two, giving a gene frequency of .035 for the mutation. This 1226 mutation represented 73% of the 124 Gaucher disease alleles in Jewish Gaucher disease patients. Accordingly we estimate that the gene frequency for Gaucher disease among the Ashkenazi Jewish population is .047, which is equivalent to a carrier frequency of 8.9% and a birth incidence of 1:450.

Introduction

Physicians often consider Gaucher disease to be rare. This disorder is caused by an inherited deficiency of the lysosomal enzyme glucocerebrosidase. Of the three types of the disease, type 1 (adult type) is by far the most common, and it is this form that is more prevalent among the Ashkenazi Jewish population than among other ethnic groups.

Accurate estimates of the prevalence of this disease have not been available because an unknown number of patients are entirely asymptomatic. Detection of the heterozygous state by enzymatic assays has not been sufficiently accurate to allow determination of the frequency of the carrier state of this disease (Desnick 1982). However, recent advances in molecular biologic techniques have provided a means for accurate detection of mutations causing Gaucher disease (Zimran et al. 1989). The most common mutation is the substitution A→G at nucleotide 1226, which produces a Asn→Ser change at amino acid 370 of the mature protein (Tsuji et al. 1988; Zimran et al. 1989). In the present study we present the results of screening for the presence of mutation 1226 in 593 unrelated normal Ashkenazi Jews and in 62 Jewish patients with Gaucher disease, and we use the data to predict the frequency of Gaucher disease within this population.

Received April 22, 1991; revision received June 5, 1991.

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0002-9297/91/4904-0018$02.00

Material and Methods

Population Studied

DNA samples were obtained from the following four populations:

Group 1.—Blood samples were taken from 168 unrelated healthy Ashkenazi Jewish volunteers largely from Israel who had a mean age of 39 years (range 18–72) (50 of these were included in a preliminary report [Zimran et al. 1990*b*]).

Group 2.—White blood cells were taken from 287 unrelated Ashkenazi Jews from New York. These samples were obtained in the course of screening for Tay-Sachs disease, at Mt. Sinai School of Medicine in New York.

Group 3.—One hundred thirty-eight cord-blood samples were taken from newborns both of whose parents were of the Ashkenazi ethnic group. These

samples were obtained at the Shaare Zedek Medical Center in Jerusalem.

Group 4. — Blood samples or cultured skin fibroblasts of all 62 unrelated Ashkenazi Jewish patients with Gaucher disease were studied at the Scripps Clinic. Thirty-five of these cases were included in a previous report (Zimran et al. 1989).

None of the individuals in groups 1–3 had any family history of Gaucher disease. All individuals in group 2 reported themselves as being healthy.

Mutation Analysis

High-molecular-weight DNA was extracted according to established methods (Maniatis et al. 1982). Detection of the 1226 mutation was performed on all samples by the PCR technique using a mismatched primer (Kumar and Dunn 1989; Beutler et al. 1990). PCR was performed in a 100-μl system containing 1 μg genomic DNA, 0.5 × PCR buffer (Kogan et al. 1987), 0.5 mM dNTPs, 5% dimethylsulfoxide (DMSO), 300 ng each oligonucleotide primer (Beutler et al. 1990), and three units *taq* polymerase (Amplitaq; Cetus, Emeryville, CA). PCR was carried out for 30 cycles, each of which consisted of denaturing at 92°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. After phenol-chloroform extraction and ethanol precipitation, one-fifth of the amplified DNA was digested with *Xho*I (Stratagene, La Jolla, CA) for 2 h according to the manufacturer's instructions. The digested DNA was then applied to a 10% polyacrylamide gel for electrophoresis and was examined by UV fluorescence after being stained with ethidium bromide. An example of such analysis is illustrated in figure 1.

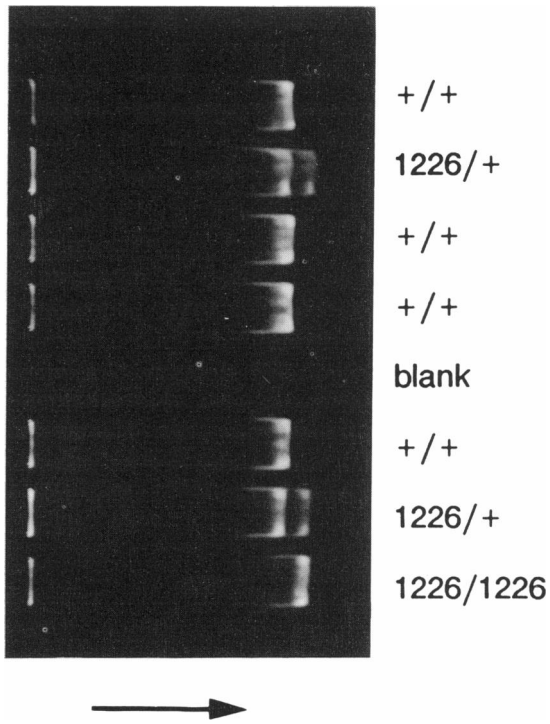

Figure 1 Detection of 1226 Gaucher disease mutation. + / + = Normal; 1226/ + = heterozygous for 1226 allele; 1226/1226 = homozygous for 1226 allele. After amplification of DNA by using the PCR, the product is digested with *Xho*I (Beutler et al. 1990). The 105-bp product of the 1226 allele is cleaved into 16-bp and 89-bp fragments. The 89-bp fragment moves farther than the normal 105-bp fragment. The 16-bp fragment is not seen on the gel.

Results

Of the 593 Ashkenazi Jewish individuals screened (groups 1–3), 37 were found to be heterozygotes for the 1226 mutation and two were found to be homozygous for it (table 1).

Differences between the gene frequencies in the three groups were not statistically significant by χ^2 analysis. The frequency of the 1226 mutation calculated from the pooled data is .0346. We were able to reexamine one of the two patients whose DNA had

Table 1

Prevalence of 1226 Mutation among Ashkenazi Jewish Samples

Group	No. of Individuals	No. of Alleles	No. of Heterozygotes	No. of Homozygotes	Total No. of 1226 Alleles	1226 Gene Frequency
1. (adult volunteers)	168	336	12	0	12	.0357
2. (Tay-Sachs screening samples)	287	574	19	2	23	.0400
3. (cord-blood samples)	138	276	6	0	6	.0217
Total	593	1,186	37	2	41	.0346
4. (Gaucher disease patients)	62	124	31	30	91	.734

revealed homozygosity for the 1226 mutation. The leukocytes from this 31-year-old male manifested only about 10% of normal acid β-glucosidase and glucocerebrosidase activity, a deficiency that is characteristic of Gaucher disease. However, he was in good general health, had no splenomegaly by physical examination or ultrasound scan, and had a normal hemoglobin level, normal white count, and normal platelet count. Skeletal X-rays were also normal. He refused marrow examination. The other subject who was found to be homozygous for the 1226 mutation could not be located.

Among the Ashkenazi Jewish patients with Gaucher disease (group 4), 30 were homozygotes for the 1226 mutation and 31 were compound heterozygotes. Hence, the frequency of the 1226 allele in them is .734.

Discussion

Accurate determination of prevalence of Gaucher disease is important for strategies of differential diagnosis and is critical to the establishment of population-based genetic screening programs. Patients with Gaucher disease can be diagnosed readily by either histological examination (usually of bone marrow) or, more appropriately, by determination of the enzymatic activity of the β glucosidase in peripheral blood lymphocytes (Beutler and Kuhl 1970; Beutler and Saven 1990). Studies based on the actual number of patients with Gaucher disease within defined Ashkenazi Jewish populations have yielded gene frequency estimates of about .01 (table 2). The detection of carriers of this disorder by enzymatic assay has not been sufficiently accurate to allow precise calculation of the gene frequency of the disease (Desnick 1982; Beutler 1988). This is the case because of the marked overlap that occurs between normals and heterozygotes for Gaucher disease. However, some estimates have been based on enzymatic assay (table 2). These have suggested a birth incidence varying from 1:640 to 1:4,000.

Recent progress in molecular biologic techniques, particularly the PCR, has allowed us to develop methods for rapid and accurate diagnosis of both Gaucher disease and the carrier state, at the DNA level (Theophilus et al. 1989; Zimran et al. 1989, 1990*b*; Beutler et al. 1990). Apart from being unequivocal and rapid, the application of DNA analysis for heterozygote detection can be performed on small quantities of blood, thus simplifying large-scale screening. Previous studies have indicated that the A→G point mutation at nucleotide 1226 of the glucocerebrosidase cDNA (Sorge et al. 1985) is the most common mutation found in patients with type 1 Gaucher disease. It is this mutation that is responsible for the high incidence of Gaucher disease in the Ashkenazi Jewish population (Theophilus et al. 1989; Zimran et al. 1989, 1990*b*), accounting for about 75% of the Jewish Gaucher disease alleles, compared with 36% among non-Jewish patients (Theophilus et al. 1989; Zimran et al. 1989).

In the present study nearly 600 unrelated Ashkenazi Jewish individuals were screened. Thus, almost 1,200 alleles were examined for the 1226 mutation, and we

Table 2

Estimates of Gene Frequency and Birth Frequency of Gaucher Disease in Ashkenazi Jewish Population

Study	Method[a]	Carrier Frequency	Gaucher Disease Frequency	Gene Frequency Estimate	Birth Frequency Estimate
Fried 1958	P		8/62,000	.011	1:7,750
Fried 1973	P		$100/10^6$	.010	1:10,000
Kolodny et al. 1982	E	17/215		.040	1:640
Grabowski et al. 1982	E	5.72/128[b]		.022	1:2,003
Matoth et al. 1987	E	14/441		.016	1:3,969
Present study	D	37/593	2/593	.047[c]	1:450[d]

[a] P = patient ascertainment; E = leukocyte enzyme assay; D = DNA analysis.

[b] Corrected for overlap with normal range.

[c] Corrected for 73% ascertainment, since only nt 1226 mutations were detected.

[d] This is actually the frequency of births at risk, since an unknown proportion of homozygotes for the 1226 mutation may never develop clinical disease.

determined its frequency to be .035 within this population. On the basis of the finding that only 73% of Gaucher disease alleles have this specific mutation, we can estimate an overall Gaucher disease gene frequency of .047, with a predicted birth incidence of 1:450, assuming that all matings are within the Ashkenazi group and that there is no consanguinity. The finding that 73% of the Gaucher disease alleles in Ashkenazi Jewish patients with Gaucher disease have the mutation at nt 1226 agrees well with the results of other studies (Theophilus et al. 1989) but may represent somewhat of an underestimate because of the mildness of the homozygous disorder. Thus, in any sample of patients with Gaucher disease, homozygotes for the 1226 allele may be underrepresented. The effect of this bias would be to decrease slightly the estimated birth incidence—although even with the limiting assumption that only the 1226 mutation existed a birth incidence of 1:835 births would be calculated. This incidence is clearly higher than the frequency with which the disease is encountered in medical practice but is supported by the detection of two homozygotes for the 1226 mutation in the 593 patients screened in the present study. Both of these patients regarded themselves as being in good health and would presumably not have sought medical attention for the treatment of their disease. One of these subjects was found to be clinically normal on detailed examination. It is not yet clear what proportion of individuals who are homozygous for the 1226 mutation will remain asymptomatic. It is well known that in some patients Gaucher disease may be detected for the first time during old age (Brinn and Glabman 1962; Beutler 1977; Berrebi et al. 1984).

Recent studies of the *Pvu*II polymorphism of the glucocerebrosidase gene have suggested the existence of a second relatively common Jewish mutation (Zimran et al. 1990*a*). If a second relatively common mutation is indeed identified, we believe that screening for this disease will be warranted among this ethnic group. Such genetic screening could be performed concurrently with the already available screening program for detection of Tay-Sachs disease.

Acknowledgments

We thank Dr. E. Shinar from the Magen-David-Adom Blood Services, Tel-Hashomer, Israel, for providing blood samples of healthy blood donors; Professor Y. Diamant, Chairman, Department of Obstetrics and Gynecology, and the nurses of the delivery room at Shaare-Zedek Medical center for providing cord-blood samples; and Brian Sipe for valuable technical assistance. This work was supported in part by NIH grants DK36639 and RR00833 (both to E.B.) and DK36729, RR00071 (both to G.A.G.), by the Sam Stein and Rose Stein Charitable Trust Fund, and by the basic Research Foundation administered by both the Israel Academy of Sciences and Humanities and the United States-Israel Binational Science Foundation (BSF), Jerusalem. This is publication 6532-MEM from the Research Institute of Scripps Clinic, La Jolla, CA.

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