An Update of Gaucher Mutations Distribution in the Ashkenazi Jewish Population: Prevalence and Country of Origin of the Mutation R496H

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ABSTRACT:

Background: Gaucher disease is the most prevalent inherited disorder among Ashkenazi Jews (carrier frequency of about 6%) and six mutations account for about 96% of their mutant alleles. Two mutations, N370S and R496H, have been reported only in mildly affected or asymptomatic patients. Due to the rarity of R496H, it was recommended that it be excluded from screening programs.

Objectives: To verify the frequency and trace the origin of Gaucher mutations in screened individuals whose Ashkenazi ethnicity was confirmed by the birthplace of their grandparents.

Methods: We conducted a retrospective analysis of the screened results for the period 2006–2011. Mutations were identified by restriction analysis, Tag-It™ detection system, Pronto® diagnostic kit and Nanogen technology (NanoChip® 400).

Results: The heterozygote frequency of eight mutations was estimated in a cohort of 16,910 alleles. Two mutations, N370S and R496H, were the most frequent in our population. However, while the occurrence of N370S carriers was similar to other reports (1:19.4), that of R496H carriers was considerably elevated (1:207). Examination of the screened individuals' ethnicity showed a significant difference in the distribution pattern of the country of origin between the carriers of these two mutations.

Conclusions: The origin pattern differences between the two groups of heterozygotes might reflect a separate geographic region of introduction for various mutations. As a result, secondary subgroups could be formed within the Ashkenazi population. This might clarify the dissimilarities in the occurrence of R496H mutation reported by various centers.

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KEY WORDS: Gaucher disease, Ashkenazi Jews, mutation distribution, R496H, mutations origin

Gaucher disease (GD) is a recessive sphingolipid storage disease caused by mutations in the glucocerebrosidase gene (*GBA*). GD is the most prevalent inherited disorder in the Ashkenazi Jewish population with a carrier frequency of about 6% [1-7]. Screening for GD carriers is still controversial since the common disease (type 1) is frequently asymptomatic and an effective treatment is available if needed. Yet, in practice, testing is offered to the Ashkenazi population by numerous centers and couples at risk are offered genetic counseling and prenatal diagnosis.

Six mutations account for about 96% of the mutant alleles among Ashkenazi Jews [1,3,8,9]. Two of them, N370S (the most frequent) and R496H, have been reported in mildly non-neurological cases or in asymptomatic patients. V394L was reported in type 3 neuropathic form in combination with L444P or RecNciI allele [10] and the other three (84insG, L444P and IVS2+1G→A) are known to be involved in the rare severe neuropathic forms of GD. Some reports suggested that the mutation R496H be excluded from the screening program due to both its rarity and the fact that it is very mild [11]. The frequency of that mutation in screened populations ranged significantly in the various studies (in contrast to N370S for example), from 1:250 (4734 samples, 9) to 0 (3336 samples, 6). The occurrence of eight mutations – N370S, R496H, 84insG, L444P, V394L, IVS2+1G→A, RecTL and RecNciI – among Ashkenazi Jewish alleles is summarized in this report. In addition, the distribution patterns of origins were analyzed among non-carriers and individuals carrying the two most prevalent mutations (R496H or N370S). Origin differences might reflect separate geographic regions of introduction for those mutations and could explain secondary subgroups within the Ashkenazi population.

SUBJECTS AND METHODS

Each individual completed a questionnaire detailing the exact origin of the four grandparents. DNA was extracted by an automatic system (MagNa Pure LC for nucleic acid purification, Roche Ltd, Switzerland). Six mutations – N370S, 84insG, L444P, IVS2+1G→A, R496H and V394L – were monitored during 2006–2011. RecTL was added to the routine screening in 2009. The studied population comprised Ashkenazi individuals

who attended the general genetic screening program. All those with a known family history of Gaucher (according to their questionnaire) were excluded from the count. During 2006–2008 the Tag-It™ mutation detection kit was used (Ashkenazi Jewish Panel, for use with Luminex® 100xMAP™ system, Tm Bioscience Corporation, Canada). In 2009 the routine tests were carried out by RFLP and the Pronto® Gaucher diagnostic kit. Nanogen technology (NanoChip® 400, Gaucher kit, Gamidor Diagnostics Ltd, Israel) has been in use since 2010. In any case of positive results to L444P the amplified fragment was sequenced to search for RecNci I recombinant mutation. All positive results were retested, in a second DNA sample, by a different method than used for the first sample.

For the restriction assays, polymerase chain reaction was designed to amplify selectively the area of each mutation in the active GBA gene. Polymerase chain reaction (PCR) conditions consisted of three cycles of 95°C for 2 minutes, 56°C for 1 min and 72°C for 1 min followed by 25 cycles of 94°C for 30 seconds, 60°C for 1 min, 72°C for 1 min and closing with 72°C for 10 minutes. Restriction assays were carried out according to the manufacturer's instructions (New England BioLabs Inc., USA). The primer sets and restriction enzymes list will be provided on request.

The distribution of allele origin (according to the questionnaires) was compared between carriers of the mutations N370S and R496H and between those carriers and non-carriers. The significance of differences between groups was analyzed by the chi-square test.

RESULTS

The study included 16,910 Ashkenazi alleles that were screened for GD carrier status during the years 2006–2011. Ashkenazi origin was verified according to the birthplace of the four grandparents: Poland, Hungary, Romania, Moldavia, Czech Republic, Germany, Austria, Belarus, the Baltic countries, Ukraine, and the European part of Russia. Our basic assumption was that mutations in the GBA gene occur very rarely in non-Ashkenazi Jewish alleles.

A GBA mutation was found in 509 individuals, implying a carrier rate of 1 in 16.6. This occurrence is within the range of other published levels [1,2,5,6,11]. The frequency of the mutations in the Ashkenazi population is detailed in Table 1. A major difference from other reports is the relatively high frequency of R496H: 1 in 207 in the examined population [4-6]. The frequencies of other mutations were similar to those in another study conducted in Israel. For example, the frequencies of 84insG and N370S in the present report (0.0021 and 0.051 respectively) did not differ significantly from those documented in the study of Fares et al., 0.003 and 0.052 [6] (chi-square with Yate's correction, $P = 0.88$ and 0.76 respectively). The mutation RecTL, which was diagnosed in 2.3% of

Table 1. Distribution of the main mutations among heterozygotes

Mutant alleles	N370S	R496H	84insG	L444P	V394L	IVS2+1
Number	433	41	18	7	7	3
Percent of carriers	85.0	8.0	3.6	1.4	1.4	0.6
Carrier frequency*	0.051	0.005	0.002	0.0008	0.0008	0.00035

Altogether, 16,910 Ashkenazi alleles were tested and 509 heterozygotes were diagnosed during 2006–2011, none of whom reported a family history of Gaucher disease
*Frequency of carriers in the tested population
Calculated confidence intervals (95%) for the carrier's frequency: N370S, 0.05-0.051; R496H, 0.003–0.0065; 84insG, 0-0.004

Table 2. Distribution of ancestor alleles of carriers and non-carriers

Genotype	Romania	Poland	Ratio
Normal alleles	390	752	0.52
N370S carriers	132	242	0.547
R496H carriers	19	17	1.11

The sorting was carried out among the grandparents' alleles of 793 non-carriers, 434 carriers of N370S and 40 carriers of R496H

the heterozygotes in our cohort, was also compatible with the published data [6] (chi-square with Yate's correction, $P = 0.32$). Nevertheless, no carriers of R496H were documented.

When the positive samples for L444P were confirmed by sequence, it was revealed that 7 of 18 samples were actually recombinant RecNciI alleles. Hence, in the present cohort, L444P as a single alteration is less frequent than reported.

In order to track a possible difference in geographic origin of the mutations, all carriers and a population of controls were sorted according to the grandparents' birth place. Only two groups of mutated alleles were large enough for statistical analysis of the prevalence among the countries of origin: carriers of N370S and R496H among alleles from Romania and Poland [Table 2].

The results are presented as ratios of allele prevalence. The ratio of "Romanian" to "Polish" alleles among N370S carriers (433 alleles) was very similar to that of 3172 control alleles: 0.547 and 0.52 respectively (chi-square, $P = 0.66$). However, the ratio among 41 R496H chromosomes was 1.11 [Table 2], a significantly different value from the control ratio (chi-square, $P = 0.0178$).

DISCUSSION

In the present study we analyzed data from 16,910 alleles of healthy members of the Israeli Ashkenazi population in order to reevaluate the frequency of Gaucher's common mutations. We found a higher than expected frequency of the mutation R496H (0.48%) as compared to those in most reports from previous screening programs, 0–0.2% [2,8,11]. In only one other large study (Allitto et al., 4734 Ashkenazi Jews) was a similar estimate

found, 0.44% [9]. Occurrence of the other mutations was not significantly different than previously documented. An excess of R496H alleles was also found in the Israeli Ashkenazi group in comparison to an Ashkenazi cohort of the International Collaborative Gaucher Group registry and it was speculated that there were probably fewer Ashkenazi alleles in the latter population [11].

Many investigations refer to Ashkenazi Jews as a single ethnic entity. The common presumption is that European Jewry originated in ancient Rome followed by continuous migrations. Due to religious and civil constraints, relatively isolated populations were formed, resulting in population substructures among Ashkenazi Jews [12-14]. The difference in ancestor origin distribution between the two heterozygote populations may reflect a different geographic region of introduction, and these variations could account for the differences in occurrence of the mutation in the various centers. Ethnic variability among Ashkenazi Jews was previously documented when analyzing various mutations: 2281delATCTGAinsTAGATTC (Bloom syndrome) and IVS4+4A>T (Fanconi's anemia) [15], and a higher carrier rate for familial dysautonomia in Jews originating in Poland [16]. In addition, a study in Australia pointed to a different incidence of Tay-Sachs mutations in Jews with a different heritage [17].

In patients, R496H has been reported only in combination with a severe mutation on the other allele [18]. It is acceptable to classify this mutation as very mild, and some studies concluded that in combination with the common Ashkenazi mutation N370S, symptoms will most likely not develop [18]. On the other hand, individuals who were diagnosed unexpectedly as compound heterozygotes (of these two mutations) actually had disease manifestations (including bone deformations) following careful clinical assessment [19]. In addition, mild Gaucher mutations were documented to cause a reduction in *GBA* expression [20].

In conclusion, due to the potential existence of genetic subgroups in the Ashkenazi population, geographic origin should be taken into account when assessing the frequency of an allele.

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> "Biographical history, as taught in our public schools, is still largely a history of boneheads: ridiculous kings and queens, paranoid political leaders, compulsive voyagers, ignorant generals, the flotsam and jetsam of historical currents. The men who radically altered history, the great creative scientists and mathematicians, are seldom mentioned if at all"

Martin Gardner (1914-2010), American popular mathematics and popular science writer. He was best known for creating and sustaining general interest in recreational mathematics, mainly through his *Scientific American* "Mathematical Games" columns