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Phenotype/Genotype Correlations in Gaucher Disease Type I: Clinical and Therapeutic Implications

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Summary

Gaucher disease is the most frequent lysosomal storage disease and the most prevalent genetic disease among Ashkenazi Jews. Gaucher disease type 1 is characterized by marked variability of the phenotype and by the absence of neuronopathic involvement. To test the hypothesis that this phenotypic variability was due to genetic compounds of several different mutant alleles, 161 symptomatic patients with Gaucher disease type 1 (>90% Ashkenazi Jewish) were analyzed for clinical involvement, and their genotypes were determined. Qualitative and quantitative measures of disease involvement included age at onset of the disease manifestations, hepatic and splenic volumes, age at splenectomy, and severity of bony disease. Highly statistically significant differences ($P < .005$) were found in each clinical parameter in patients with the N370S/N370S genotype compared with those patients with the N370S/84GG, N370S/L444P, and N370S/? genotypes. The symptomatic N370S homozygotes had onset of their disease two to three decades later than patients with the other genotypes. In addition, patients with the latter genotypes have much more severely involved livers, spleens, and bones and had a higher incidence of splenectomy at an earlier age. These predictive genotype analyses provide the basis for genetic care delivery and therapeutic recommendations in patients affected with Gaucher disease type 1.

Introduction

Gaucher disease type 1 is the most prevalent lysosomal storage disease and the most common Jewish genetic disease (Zimran et al. 1991; Beutler and Grabowski, in press). The disease results from numerous mutations at the acid β-glucosidase locus on chromosome 1, which lead to defective activity of this enzyme (reviewed in Grabowski et al. 1990; Beutler 1991; Beutler and Grabowski, in press). The resultant accumulation of glucosylceramide within cells of monocyte/macrophage origin leads to the visceral manifestations of the disease (Lee 1982). Gaucher disease has been classified into three major clinical variants which are distinguished by the absence (type 1; nonneuronopathic) or presence (types 2 and 3; acute and subacute neuronopathic, respectively) of primary central nervous system disease (Beutler and Grabowski, in press). Significant phenotypic heterogeneity occurs within each variant, but it is the least variable in type 2 disease which leads to death by ~2 years of age.

Although over 25 mutant alleles have been delineated (reviewed in Grabowski et al. 1990; Beutler and Grabowski, in press), only four occur with substantial frequency in the general population and, particularly, in the Ashkenazi Jewish population. These include the mutations encoding N370S, 84GG, L444P, and IVS 2^{+1} (Tsuji et al. 1987, 1988; Beutler et al. 1991*a*, 1992; Zimran et al. 1991; He and Grabowski 1992). The 84GG and IVS 2^{+1} alleles indicate nonsense mutations resulting from a G insertion at nucleotide 84 in the cDNA or a G → A transition at the exon2/intron2 splice junction, respectively. Recent studies have indicated that these four mutant alleles account for ~50%–70% of the disease alleles in affected patients from unselected populations and ~89%–95% of Gaucher mutant alleles in the Ashkenazi Jewish population (Beutler et al. 1991*a;* He and Grabowski 1992) (table 1). Moreover, molecular studies indicate that about $^1/_{10}$–$^1/_8$ Ashkenazi Jews are carriers of a Gaucher

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Table 1

Frequency of Jewish Gaucher Disease Mutations in 258 Jewish Patients

Mutation	No. of Alleles	Percent
N370S	369	71.5
84GG	60	11.6
L444P and XOVR[a]	23	4.44
IVS 2^{+1}	11	2.2
V394L[b]	5	.96
Other[c]	48	9.3

[a] XOVR is a fusion gene containing multiple point mutations, including L444P. Adapted from present data and Beutler et al. (1992).

[b] Described in Theophilus et al. (1989*a*).

[c] Includes unidentified, rare, and private alleles in this population.

disease allele (Zimran et al. 1991), thereby making this disease a prime candidate for population-based heterozygote screening. However, the markedly variable phenotype in affected patients with Gaucher disease type 1 has made genetic counseling and recommendations for specific therapy problematic. Also, the serendipitous discovery of asymptomatic, acid β-glucosidase–deficient individuals with the N370S homozygous genotype makes prognostication of disease severity difficult. Prenatal diagnostic options and the availability of effective enzyme therapy for this disease (Barton et al. 1991; Beutler et al. 1991*b;* Fallet et al. 1992) provide the rationale for the need to develop prognostic tests for severity of involvement.

To gain insight into the relationship between genotype and phenotypic involvement in type 1 Gaucher disease, 161 patients were evaluated by clinical measurements, and correlations were made with genotype. The results of these studies provide the information for prediction of the severity of Gaucher disease and guidelines for the use of genotype data in the institution of therapy and for the provision of genetic care to affected families.

Subjects, Material, and Methods

One hundred sixty-one patients with Gaucher disease were evaluated on the General Clinical Research Centers of the Mount Sinai School of Medicine or the Children's Hospital Medical Center. The patients' ages at the time of the study were from 2–84 years. Seventy-two patients were female and 89 were male. Six patients had intercurrent illnesses, unrelated to Gaucher disease, including chronic renal failure (1), posthepatitic diseases (4), and acquired atherosclerotic myocardial disease (1). All other patients were devoid of other chronic illnesses. All patients had been followed or had medical records for a 1–30-year period and had normal intellectual and/or developmental studies. None of the patients had oculomotor apraxia, which is indicative of neuronopathic involvement (Sidransky et al. 1992). The age at onset of disease manifestation was established by medical records and/or patient recollection of when they were first diagnosed. When both criteria were available ~90% concordance was observed between medical record data and patient recollection. All patients were confirmed as having acid β-glucosidase deficiency in cultured fibroblast and/or peripheral blood leukocyte assays using artificial and natural substrates according to a method described by Grabowski et al. (1985). To avoid ascertainment biases, affected siblings were excluded from the analyses so that only the index cases were included in the statistical studies. This exclusion should eliminate the potential for ascertainment bias of age at onset because of detection by family studies.

Liver and splenic volumes were determined by computed tomography (CT) scan according to a method described by Tarao et al. (1989) and were referenced to body weight as 2.5% and 0.2% of body weight for the normal liver and spleen volumes, respectively (Ludwig 1979, pp. 676–685). The liver or splenic volumes obtained by quantitative CT scan were converted to masses of the respective organ by assuming 1 cc/g of liver or spleen tissue. The *n*-fold increases were calculated by dividing organ masses by the predicted normal mass for the liver or spleen. Bone severity was determined on a quantitative scale by using radiographic procedures described elsewhere (Beighton et al. 1982; Hermann et al. 1986), and patients were assigned the numerical score for the stage of involvement (table 2). Statistical analyses were conducted with the Systat software programs (Systat).

Analyses of genomic DNA for the N370S, 84GG, L444P, and IVS 2^{+1} mutations, as well as for several other rare alleles, were conducted using oligonucleotide-specific hybridization or specific restriction endonuclease digestion of PCR-amplified genomic DNA from affected patients. Genomic DNA for analysis was prepared from 500 µl of whole blood by the rapid boiling method or on purified DNA from cultured fibroblasts or lymphoid lines. The four common mutations were detected by PCR amplification (He and Grabowski 1992) and direct mutation detection at natural or created restriction sites (Tsuji et al. 1987; Beutler et

Table 2

Radiologic Stages of Skeletal Lesions in Gaucher Disease Type I

Stage	Type of Lesion/Site Involved	Radiographic Appearance
1	Diffuse osteoporosis/ tubular bones and vertebrae	Coarse trabecular pattern of osteoporosis
2	Medullary expansion/ femoral, long bones, ribs	Loss of normal concavity above femoral condyles; Erlenmeyer flask deformity
3	Localized destruction (osteolysis)/long bones	Small erosions (well defined or moth eaten); cortex rarefied and endosteal notching; ground-glass veiling
4	Ischemic necrosis, sclerosis, osteitis/ long bones	Patch densities and erosions; serpiginous sclerotic streaks; layered periostitis; sequestra
5	Diffuse destruction; epiphyseal collapse; osteoarthrosis/ hips, shoulders, vertebrae, sacroiliac joints	Flattening or irregular destruction of femoral or humeral heads; mixed lytic and sclerotic floci; larger "soap bubble" pattern

SOURCES.—Adapted from Hermann et al. (1986) and Beighten et al. (1982).

al. 1991*a;* He and Grabowski 1992). For detection of the N370S mutations, a mismatched primer strategy (Beutler et al. 1990) that creates an *Xho*I site in the mutant sequences was used with minor modifications. Thirty cycles of PCR amplification were performed as follows: 94°C for 15 s, 59°C for 15 s, and 72°C for 30 s. After digestion with *Xho*I, the normal and mutant products were resolved on 12% acrylamide gels. A mismatched primer protocol was also employed for detection of the 84GG mutation (Beutler et al. 1991*a*). Thirty cycles of amplification were performed as described above, followed by digestion with *Bsa*BI and electrophoresis on 12% acrylamide gels. The L444P (Tsuji et al. 1987) mutation creates a *Nci*I restriction site which was detected by electrophoresis after *NciI* digestion of the appropriate PCR-amplified structural gene segments. The IVS 2^{+1} (Beutler et al. 1992; He and Grabowski 1992) mutation obliterates an *Hph*I site and was detected after PCR amplification of genomic DNA using the following primers: 5′ primer TCCACATCG-GAAGCCGGAAT and 3′-primer AACAGAGATA-GACTCTGGTTC. Electrophoresis on 1% agarose gels was conducted after *Hph*I digestion. Other rare alleles were determined by sequence-specific hybridization according to methods described elsewhere (Latham et al. 1990, 1991).

Results

Only patients with symptomatic Gaucher disease were evaluated for these studies. These patients had significant symptoms of hepatic and/or splenic enlargement, hematologic abnormalities, or bone disease. In addition, no patients with asymptomatic acid β-glucosidase deficiency (Gaucher disease) or those serendipitously diagnosed by family studies were included in this evaluation. Therefore, the data are heavily biased toward patients with symptoms and signs. In our experience, no patients with genotypes other than N370S homozygosity have been asymptomatic and, thus, the data for N370S homozygotes are biased toward the more symptomatic patients with this genotype.

Shown in table 3 are the genotype/phenotype correlations from the analyses of these 161 patients. The means, ranges, SDs, and numbers of patients evaluated are presented for each of the categories. The same data are shown graphically in figures 1*A, B, C,* and *D* for each of the categories and indicate the distribution of patient results in the four analyses. The pairwise comparisons between each of the clinical parameters obtained from the N370S homozygotes and those from patients with each of the other genotypes were highly significant ($P < .005$). For example, the means for the age at onset and splenic volumes in patients with the N370S/84GG genotype were 9.09 years and 33.7-fold greater than normal. The corresponding values in the N370S/N370S patients were 34.68 years and ~8.6-fold. The splenectomy rate in the N370S homozygotes was 10%. In comparision, these rates were 2.4–4.8-fold greater for patients with the N370S/84GG (46%), N370S/L444P (24%), and N370S/? (48%) genotypes. For each clinical parameter the pairwise comparisons between the N370S/84GG, N370S/L444P, and N370S/? groups were not significant at the $P < .01$ level. However, the splenic volumes were significantly larger for the N370S/84GG patients at the $P < .05$ level. Although very significant differences were observed between the clinical parameters for the N370S homozygotes and those for the other genotypes, the distribution data indicated a substantial variation in the age at onset within each genotype. For example, seven N370S homozygotes had ages at onset below 10 years

Table 3

Genotype/Phenotype Correlations in Jewish Gaucher Disease

Genotype	Age at Onset (years)	Bone (stage = 0–5)[a]	Liver Enlargement[b]	Splenic Enlargement[b]	Age at Splenectomy (years)
N370S/N370S:					
Mean	34.68	2.03	1.22	8.58	56
Range	3–73	1–4	.79–2.14	1.8–25.1	26–69
Number	59	37	35	33	6
SD	19.1	.763	.304	15.5	15.5
N370S/84GG:					
Mean	9.09	3.53	2.67	33.7	21.18
Range	2–28	1–5	1.0–4.12	10.2–62	5–57
Number	37	26	15	9	17
SD	7.57	1.14	.93	13.4	13.4
N370S/L444P:					
Mean	16.9	3.11	2.11	22.23	25
Range	2–35	1–5	1.7–2.69	6.89–43.3	2–44
Number	17	9	10	9	4
SD	11.3	1.23	.33	12.1	19.1
N370S/IVS 2^{+1} or ? and ?/?[c]					
Mean	14.28	3.03	2.08	23.83	26.1
Range	1–45	1–5	.74–3.48	7.4–75	5–69
Number	48	38	28	7	23
SD	11.38	1.19	.68	18.06	18.24

[a] See table 2 for explanation of stages. 0 = normal.
[b] Value indicates *n*-fold increase over normal.
[c] A question mark (?) indicates unidentified or rare alleles in the Gaucher disease population.

and ten N370S/? patients had ages at onset above 30 years. Such skewed distributions also were observed for the other categories of comparison, although they were less pronounced. With the N370S/? genotype, this would be expected, since a large number of rare alleles with different effects on the enzyme are present in this group. However, in the N370S/N370S, N370S/84GG, and N370S/L444P groups the skewness suggests other modifying factors (genetic or environmental) which altered the expression of the phenotypes.

Other parameters of assessment also included hemoglobin levels, platelet counts, acid phosphatase levels, angiotensin converting–enzyme levels, chemical assessments of liver function, as well as pulmonary function studies. These parameters were highly variable and did not reach statistical significance at the $P < .05$ level for any of the genotypes tested, although the hematologic findings were generally less pronounced in the N370S homozygotes.

A questionnaire was completed by the patients to assess their degree of involvement. In general, the results suggested that patients with N370S homozygosity viewed their disease as less severe than did those patients with the other genotypes. These were not used in any of the assessments since these parameters, as well as many of those suggested by Zimran et al. (1989) for a severity index, are subjective and difficult to interpret.

In addition to the analyses described above, we have reviewed the literature on genotyping in Gaucher disease patients and have catalogued those results in table 4. Although assessment could not be made as to the severity of involvement by the same clinical categories as evaluated in this study, assignment of the variant type (type 1, 2, or 3) of Gaucher disease and its relationship to genotype was conducted. In this analysis, we attempted to exclude all those data that appeared to be duplicate results obtained by analyzing the same patients and only included those patients for whom there were clear data placing them in each of the types. As can be appreciated from table 4, the N370S allele, either in the homozygous or heterozygous state, occurs only in the nonneuronopathic Gaucher type 1 population. In comparison, the L444P allele occurred in types 1, 2, and 3, but homozygosity occurred almost exclusively in types 2 and 3. One report indicates that homozygosity for L444P was associated with nonneurono-

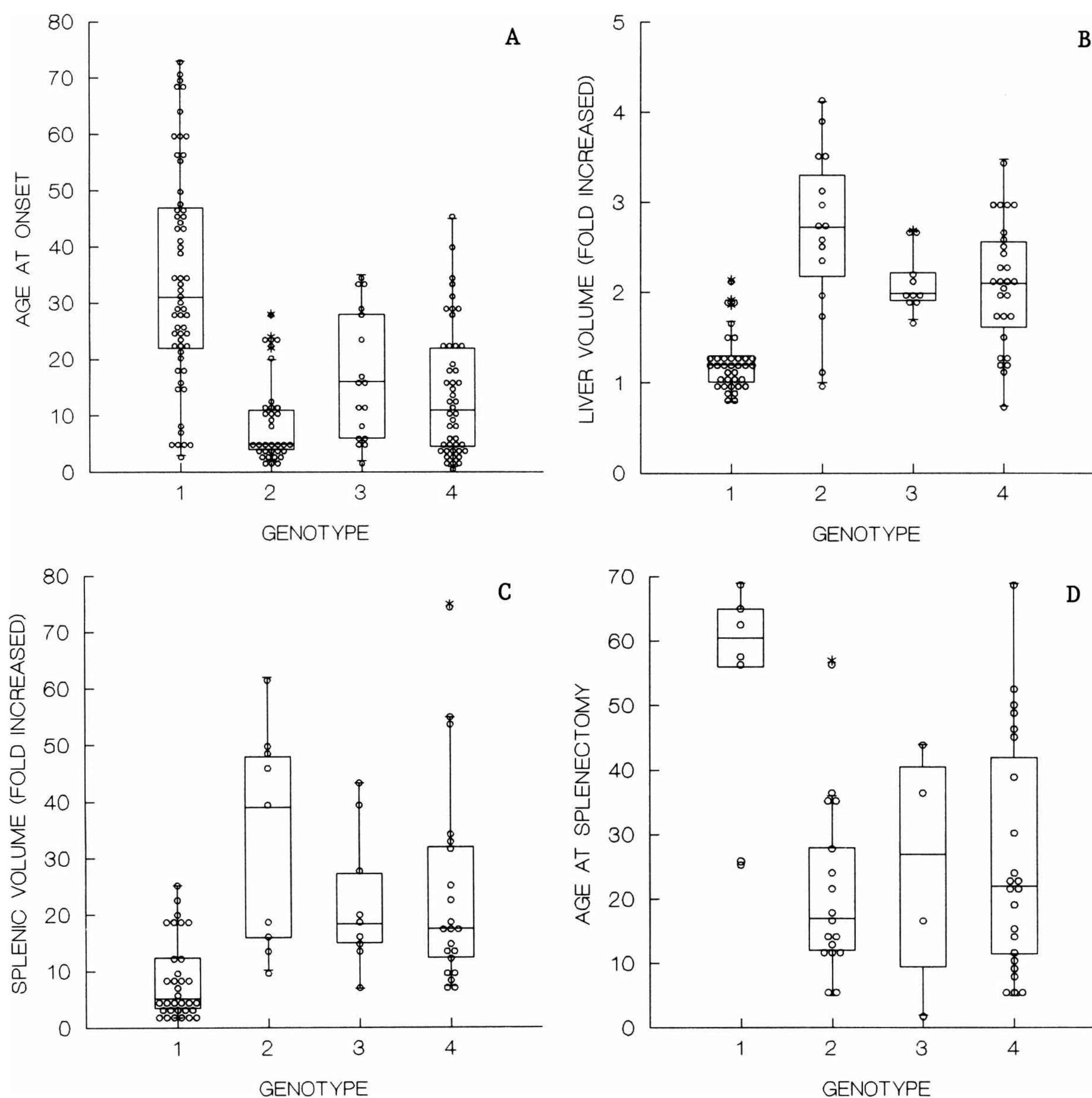

Figure 1 Distribution of values for age at onset of disease (*A*), liver volume (*B*), splenic volume (*C*), and age at splenectomy (*D*), for each genotype in Gaucher disease type 1. Genotypes 1, 2, 3, and 4 refer to N370S/N370S, N370S/84GG, N370S/L444P, and N370S/?, respectively. The horizontal lines within the boxes indicate the median value for each clinical parameter. The boxes and vertical lines represent the first- and second-quartile ranges. Asterisks indicate values beyond the third quartile. Formal statistics are summarized in table 3.

pathic disease in a Japanese teenager (Masuno et al. 1990). In addition, some individuals, who were shown to be homozygous by allele-specific hybridization or restriction endonuclease studies for the L444P alleles and had type 2 disease, were subsequently shown to have at least one allele with additional mutations representing a pseudogene conversion-type allele (Hong et al. 1990; Latham et al. 1990). The remaining category of unknown alleles includes the additional 25 known rare, as well as unknown, alleles (Beutler and Grabowski, in press).

Discussion

Genetic counseling and provision of medical care to patients affected with Gaucher disease has been particu-

Table 4

Frequency of Mutant Alleles among the Gaucher Disease Variants

Variant	Frequency of Mutant Allele		
	N370S	L444P	Other
Type 1	439/704 (62%)	79/635 (12.4%)	107/352 (30%)
Type 2	0/30 (0%)	55/52 (48%)	12/18 (67%)
Type 3[a]	0/69 (0%)	55/80 (68.8%)	8/24 (33%)

NOTE.—Adapted from Tsuji et al. 1987, 1988; Theophilus et al. 1989*a*, 1989*b*; Zimran et al. 1989; Firon et al. 1990; Latham et al. 1990; Choy et al. 1991; Grace et al. 1991; Latham et al. 1991; Sidransky et al. 1992.

[a] Does not include the Norbottnian type 3 population.

larly difficult because of the marked phenotypic variation of the disease types (Beutler and Grabowski, in press). In an effort to develop methods for the prediction of severity within a type, investigators attempted to use either absolute levels of enzymatic activity or physicokinetic properties of the residual enzyme in Gaucher disease cells to predict the classification of the disease (e.g., see Grabowski et al. 1985; Glew et al. 1988). Although in broad terms these efforts failed, the biochemical studies have provided insight into the molecular etiologies of Gaucher disease (Grace et al. 1990, 1991; Ohashi et al. 1991). The advent of therapies has made the genetic counseling and provision of care to families with Gaucher disease even more time consuming and difficult. Enzyme therapy has proved highly efficacious in Gaucher disease type 1 (Barton et al. 1991; Beutler et al. 1991*b*; Fallet et al. 1992), and bone marrow transplantation has been used in selected cases with good results (Starer et al. 1987; Tsai et al. 1992). Indications are that early intervention with enzyme therapy may lower the costs and prevent the development of major disease manifestations, but long-term studies are required for substantiation (Grabowski 1992). However, because of the cost, as well as theoretical (e.g., HIV and HBV contamination) and actual (antibody reactions) toxicity concerns, only patients with established major symptoms and signs of Gaucher disease have been treated to date. In comparison, people with minimal manifestations early in life are being followed and await the development of major changes that would be required for the initiation of therapy. Because of both the destructive (and perhaps irreversible) nature of the bony disease and the irreversibility of some tissue damage due to the Gaucher disease process (Beutler and Grabowski, in press), it may be beneficial to institute earlier therapy to prevent the onset and occurrence of these manifestations in individuals who would develop severe disease.

The present studies indicate that by using five clinical parameters of Gaucher disease involvement (age at onset, bony severity, liver and splenic enlargement, and age at splenectomy), the degree and severity of involvement of symptomatic patients with Gaucher disease type 1 can be predicted from their genotypes with a high degree of confidence. Such prognostic information will be useful not only in genetic counseling of families but also for the potential institution of therapy early in life for the prevention of the disease manifestations. The data presented here demonstrate that symptomatic patients with genotypes other than N370S homozygosity will develop much more severe disease of their bone, liver, and spleen, at an earlier age. The data of Beutler (1992) for age at onset were about 4 years lower for each genotype than the results from our population. When the present data and those of Beutler (1992) are used, the median ages of onset of disease for symptomatic patients were, for N370S/N370S (30.8 years; n = 120), for N370S/84GG (7.4 years; n = 62), and for N370S/L444P (13.5 years; n = 30). On the basis of these results in Gaucher disease type 1, genetic counseling and provision of medical care to at-risk families must now include informed discussions of the correlations between genotype and potential phenotypic involvement. In addition, prenatal diagnostic assays for Gaucher disease should include genotyping as well as enzymatic assays of acid β-glucosidase activity.

Review of the literature demonstrates that the major variants of Gaucher disease, i.e., nonneuronopathic (type 1) or neuronopathic (types 2 and 3), also can be predicted from the genotype, with a reasonable degree of accuracy. In affected patients the presence of the N370S allele apparently excludes the occurrence of a neuronopathic phenotype. In comparison, the L444P homozygous genotype is highly associated with the neuronopathic disorders (types 2 or 3), neither of which has been shown to be amenable to current therapy. Bone marrow transplantation in one type 3 patient may have altered the central nervous system course in one Swedish patient (Erikson et al. 1990). A few cases of very severe, apparently nonneuronopathic phenotypes have been reported to have this genotype (Theophilus et al. 1989*a*, 1989*b*; Masuno et al. 1990; Sidransky et al. 1992). These findings indicate the need for vigilance in the diagnosis, follow-up, and genotyping of such patients to establish the natural history of their disease. Similarly, since the N370S, L444P, 84GG, and IVS 2^{+1}

alleles account for about 50%–70% of all Gaucher disease alleles in unselected populations, many, but not all, genotype assignments can be made in at-risk families. However, in families outside the Ashkenazi Jewish population, many rare or private alleles are present, and genotyping of affected children may not provide predictive information at present. In particular, demonstration of heterozygosity for a L444P allele and an unknown or rare allele in an affected child provides no information as to the future development of neuronopathic involvement.

The above discussion is predicated on the delivery of informed genetic counseling and genetic care to patients affected with Gaucher disease. The use of genotype/phenotype correlation data must be presented to patients and affected families in a highly controlled, carefully designed infrastructure, in centers staffed by knowledgeable, experienced medical genetic personnel. In addition, physicians knowledgeable about Gaucher disease must be available, since the broad variation in phenotypic manifestations within the genotypes must be addressed when providing care and counseling to affected patients and their families. Furthermore, as the base of a well-studied patient population expands, exceptions to the above genotype/phenotype correlations may be detected. The rate of such exceptions will have significant impact on the information provided to at-risk and affected families. As a prototype for genetic susceptibility counseling, the complex therapeutic, personal, and psychosocial issues will need to be addressed and will require a restructuring of current genetic care delivery to accommodate such. The implications for the expanding list of other life-threatening or chronic, debilitating inherited diseases —e.g., cystic fibrosis, for which protein and/or gene-based therapies will be developed—necessitates the expansion and reeducation of genetic care providers.

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