Gaucher Disease

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Keywords
Gaucher disease · Genotype/phenotype correlation · Enzyme replacement therapy · Pharmacological chaperone therapy · Substrate reduction therapy

6.1 Case Report

A 7-month-old infant was admitted to the hospital because of afebrile convulsion. The patient was born with a normal pregnancy at full term. The delivery was uncomplicated. He developed normally until 3 months of age. However, he manifested stridor and poor feeding at 4 months old and exhibited retroflexion of the neck and strabismus. Feeding problems and difficulty in handling salivary secretion appeared at 6 months old. He manifested tonic-clonic convulsion without fever. Since convulsion continued for more than 30 min, he was transferred to the emergency room.

His body weight, height, and head circumference were 6.2 kg (−2.2SD), 67.0 cm (−0.8SD), and 41.1 cm (3 percentile), respectively. He could control his head, but he could not roll over or sit up. Facial appearance was normal. Severe stridor and retraction were noted upon chest exam. No heart murmur was audible. The abdomen was swollen and moderate splenomegaly was pointed out. The patient's liver was palpable at 3 cm under the right costal margin. Additionally, the tip of spleen was palpable between the level of the umbilicus and the pelvic rim without extension to the right side of the abdomen. On neurological examination, he showed tonic posture, trismus, and spasticity.

Hemoglobin level, white blood cell, and platelet counts were 10.8 g/dl, 6700 /μl, and 9.0×10^4/μl, respectively. On blood biochemistry, aspartate aminotransferase (AST; normal range is 25–80 U/l), alanine aminotransferase (ALT; normal range is 10–60 U/l), lactate dehydrogenase (LDH; normal range is 370–820 U/l), and total bilirubin (normal range is 0.2–1.4 mg/dl) were 32 U/l, 26 U/l, 380 U/l, and 0.2 mg/dl, respectively. The levels of acid phosphatase (ACP) and angiotensin-converting enzyme (ACE) were 88 IU/l/ and 98 IU/l, respectively. Normal ranges of ACP and ACE are less than 14 IU/l and 7–30 IU/l, respectively.

6.2 Diagnosis

6.2.1 Clinical Diagnosis

A clinical hallmark of Gaucher disease (MIM #230800) is hepatosplenomegaly. Based on the

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Table 6.1 Clinical phenotypes of Gaucher disease

	Type 1 non-neuronopathic form	Type 2 acute neuronopathic form	Type 3 subacute neuronopathic form
		Neuronopathic Gaucher disease	
Onset	Child ~ adult	Neonate/infant	Child ~ adolescence
Neurological manifestation	-	+++	+~++
Hepatosplenomegaly	- ~ +++	+	+ ~ +++
Bone involvement	- ~ +++	-	- ~ +++
Prognosis	Good	Poor	Various

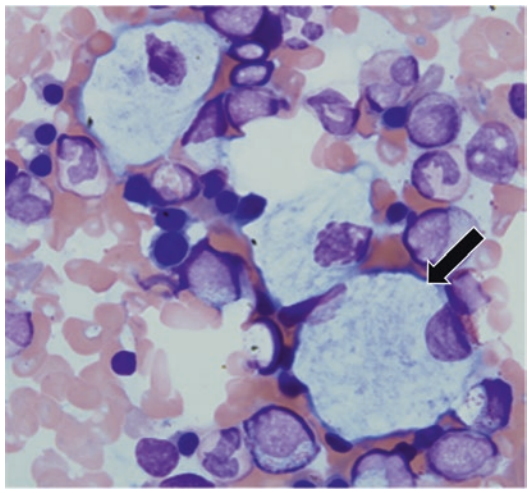

Fig. 6.1 Gaucher cell
The size of abnormal cell (arrow) is approximately 80 μm. The cytoplasm has "wrinkled tissue paper" appearance and the nucleus is eccentric

presence and rate of progression of neurological symptoms, Gaucher disease is classified into three clinical phenotypes (Table 6.1). In type 1 Gaucher disease, patients have no neurological manifestations and chronic course. A clinical feature of type 1 Gaucher disease is variability in age of onset, severity, and progression. Type 2 Gaucher disease is characterized by severe and progressive neurological deterioration and is either fatal at birth or within 2–3 years. Patients with type 3 Gaucher disease have neurologic symptoms with later onset and a more chronic course than that observed in type 2 disease.

Thrombocytopenia is the common peripheral blood abnormality. Anemia is usually mild. Leukopenia also occurs in some patients. These hematologic manifestations are probably due to a combination of increased splenic sequestration and decreased production because of replacement of the bone marrow by Gaucher cells (Fig. 6.1). Since Gaucher cells contain ACP and macrophages produce ACE, these enzymes are elevated in patients with Gaucher disease. The skeletal manifestation of Gaucher disease can be totally debilitating. The "Erlenmeyer flask deformity," which is the expanded cortex of the distal femur, is a common radiographic finding. "Bone crises," pathologic fracture of the long bone, vertebral collapse, and avascular necrosis of the femoral neck can be seen in type 1 patients with a severe phenotype. Involvement of the central nervous system can be seen in patients with type 2 and type 3 Gaucher disease (neuronopathic Gaucher disease). Oculomotor abnormalities are often the first manifestations with the appearance of bilateral, fixed strabismus or oculomotor apraxia. Patients may also show hypertonia of the neck muscles with extreme retroflexions of the neck; bulbar signs, limb rigidity, and seizures occur (Fig. 6.2).

When you see a patient with hepatosplenomegaly, Niemann-Pick disease and cholesteryl ester storage disease should be ruled out. Other possible differential diagnoses are leukemia, osteomyelitis, and Perthes disease when a patient manifests skeletal manifestations.

6.2.2 Biochemical Diagnosis

Gaucher disease is a lysosomal storage disorder caused by the deficiency of glucocerebrosidase (GBA) activity and consequent storage of gluco-

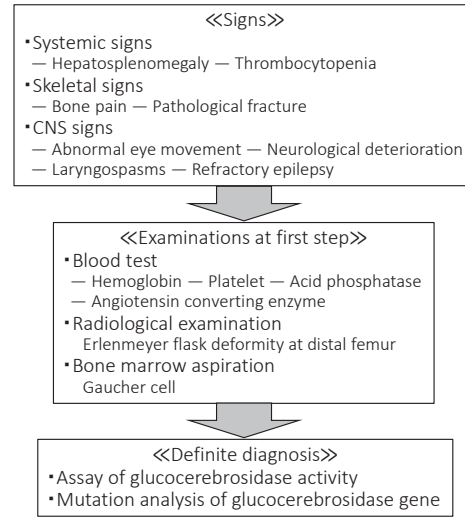

Fig. 6.2 Diagnostic flow for Gaucher disease Physicians suspect Gaucher disease based on clinical findings, and then first step examinations will be done. Definite diagnosis can be made by enzyme assay, and less than 10% value of normal enzyme activity leads the diagnosis of Gaucher disease. Mutation analysis of GBA gene is confirmative

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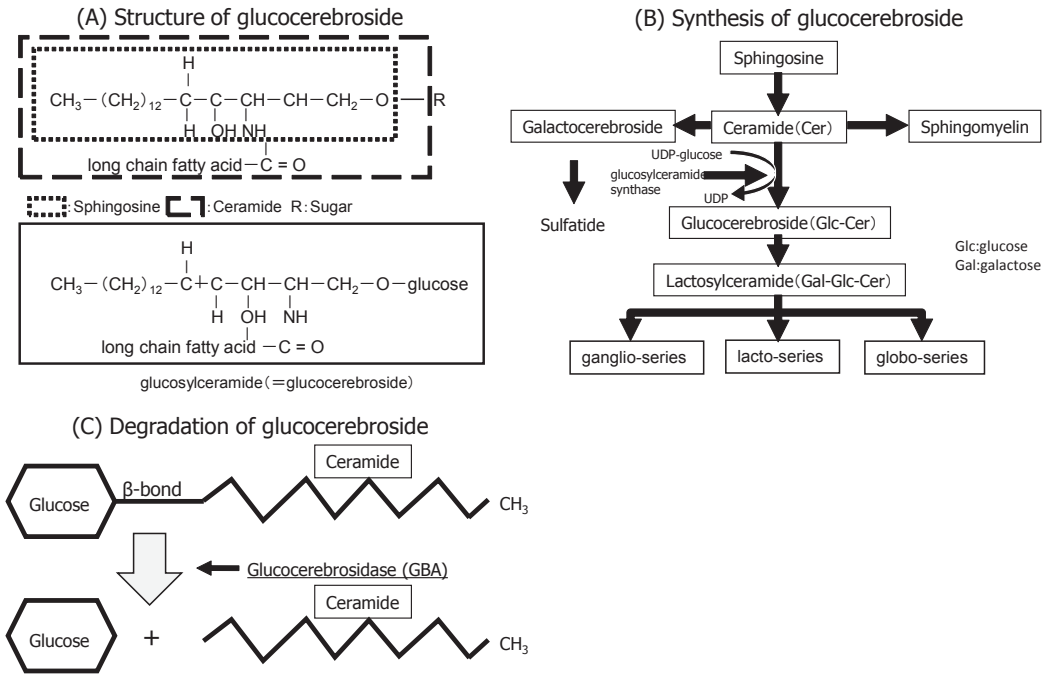
cerebroside in the cells of the monocyte/macrophage lineage (Figs. 6.3 and 6.4). Artificial, water-soluble substrates for the β-glucosidase enzyme are very useful in the diagnosis of Gaucher disease. 4-Methylumbelliferyl-β-glucoside (4MU-β-glucoside) can be used as a substrate to make the diagnosis of Gaucher disease. However, since the activity of the β-glucosidase in monocytes, lymphocytes, and granulocyte is different, it is better to separate these cells for enzyme assays. The enzyme activity in cultured skin fibroblasts is stable and reliable. There is the overlap of enzyme activity between the normal subjects and heterozygotes, and enzyme-based diagnosis is not useful in differentiating neuronopathic and non-neuronopathic Gaucher disease.

6.2.3 Pathological Diagnosis

The pathological characteristics of Gaucher disease are the presence in various tissues of lipid-engorged cells, referred to as a Gaucher cell, due to their hallmark appearance. A typical Gaucher cell contains one or more nuclei and cytoplasm with a striated, fibrillary, or tubular pattern, which is so-called wrinkled tissue paper or crumpled skin (Fig. 6.1). Gaucher cells are distributed systemically; however, they are mainly found in the spleen, sinusoids of the liver, bone marrow, and parenchyma of the lymph nodes. Their distribution and origin are important in the pathophysiology of Gaucher disease. However, very similar cells, pseudo-Gaucher cells, are found in other disorders such as chronic granulocytic leukemia, thalassemia, multiple myeloma, Hodgkin disease, and plasmacytoid lymphomas. Because biochemical and molecular diagnosis are specific and less invasive, pathologic diagnosis of Gaucher disease is supportive.

6.2.4 Molecular Diagnosis

There have been over 300 reported causative mutations in the GBA gene. The N370S mutation and c.84–85insG mutation are common in Jewish patient population (Fig. 6.5). Mutations N370S, L444P, c.84–85insG, and IVS2 + 1G → A account for more than 95% of the mutated alleles in Ashkenazi Jewish patients. The N370S mutation is a G to A transition at nucleotide 5841 of the GBA gene, substituting serine for asparagine. The c.84–85insG mutation is an insertion of an extra G at nucleotide 84 of the cDNA. Due to the insertion of an extra nucleotide, shift of the protein translation frame occurs, resulting in prema-



Structure of glycosphingolipids is shown in figure (A). Glucosylceremide (glucocerebroside) is synthesized from ceramide and UDP-glucose by glucosylceramide synthase (B) and it is made in the Golgi complex. Glucecerebroside is degraded by GBA in the lysosome (C).

Fig. 6.3 Structure, synthesis, and degradation of glucocerebroside The structure of glycosphingolipids is shown in figure (**a**). Glucosylceramide (glucocerebroside) is synthesized from ceramide and UDP-glucose by glucosylceramide synthase (**b**), and it is made in the Golgi complex. Glucocerebroside is degraded by GBA in the lysosome (**c**)

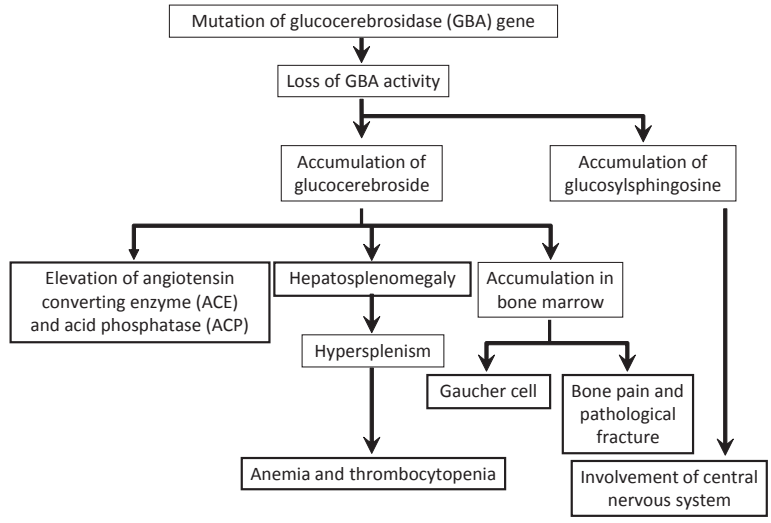


Fig. 6.4 Pathophysiology of Gaucher disease GBA gene mutation causes the loss of enzyme activity, resulting in accumulation of glucocerebroside and glucosylsphingosine. These biochemical abnormalities lead the clinical manifestations

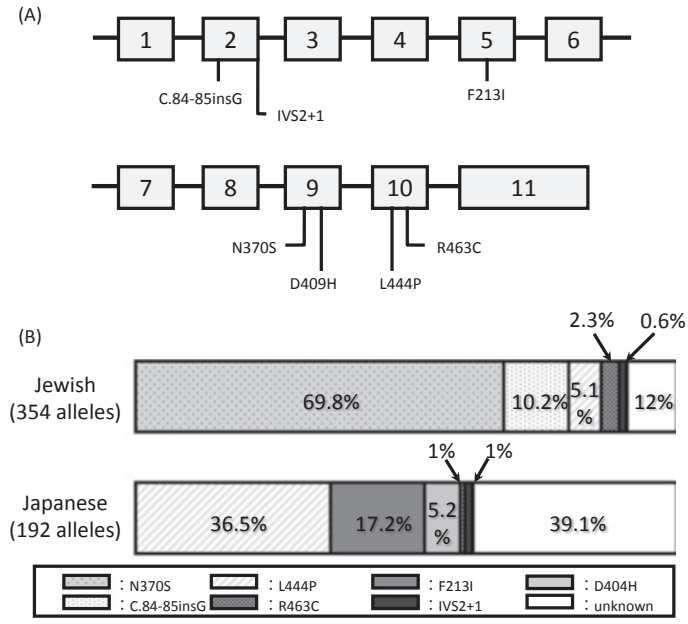


Fig. 6.5 Structure of GBA gene and location of common mutations (**a**) and mutation prevalence in Jewish and Japanese patients with Gaucher disease (**b**)

ture termination. If Gaucher disease is suspected clinically in a Jewish patient, molecular diagnosis is useful to confirm the diagnosis because of characteristic mutation prevalence described above. However, since the prevalence of mutations is different among various ethnicities, particularly in the Japanese population, a DNA-based diagnosis is less helpful (Fig. 6.5). Unlike an enzyme-based diagnosis, mutation analysis can distinguish a heterozygote from a normal individual and offers the possibility of predicting the clinical phenotype. The presence of the N370S mutation can exclude the possibility of neuronopathic Gaucher disease, and it is highly associated with type 1 patients with mild phenotypic expression. Homozygotes for the L444P mutation all have severe visceral involvement and usually have neurological manifestations.

6.2.5 Diagnosis of This Case

This case is an infant with neurological involvement, splenomegaly, thrombocytopenia, and elevation of ACP and ACE. Bone marrow aspiration was carried out, and characteristic Gaucher cells were found (Fig. 6.1). To confirm the diagnosis, an enzyme assay using cultured skin fibroblast was performed, and GBA activity was determined to be 56 nmol/hour/mg protein. This value was 5.1% of control fibroblasts, suggesting that the diagnosis of this patient is Gaucher disease. Mutation analysis using white blood cells was done, and his genotype was L444P/RecNciI (refer to the Molecular basis of GBA gene in the following section). A diagnostic flow chart is shown in Fig. 6.2. Based on the data described above, this patient can be diagnosed as type 2 Gaucher disease.

6.3 Biochemical and Molecular Perspectives

6.3.1 Biochemistry

Glucocerebroside is synthesized from ceramide and UDP-glucose by glucosylceramide synthase, and it is degraded to ceramide and glucose by GBA (Fig. 6.3). GBA is one of the lysosomal enzymes and is a glycoprotein. It has a molecular weight of about 65KDa. There are five consensus sequences for glycosylation sites. Terminal sugars are important to target the monocytes/macrophage in which glucocerebroside accumulates (see the enzyme replacement therapy (ERT) in the

Treatment section). GBA gene mutations that lead to the loss of enzyme activity result in the accumulation of glucocerebroside in the lysosomes of macrophages. This biochemical abnormality causes clinical manifestations such as hepatosplenomegaly, anemia, thrombocytopenia, and involvement of bone tissue. As a result of accumulation of glucosylsphingosine, a lysoderivative of glucocerebroside, patients with neuronopathic Gaucher disease manifest neurological symptoms. The pathophysiology of Gaucher disease progression is represented in Fig. 6.4.

6.3.2 Molecular Basis of GBA Gene

The functional GBA gene is about 7Kb in length and consists of 11 exons. The active site of the GBA enzyme is encoded by exons 9 and 10. Seven common mutations (c.84–85insG, IVS2 + 1G → A, F213I, N370S, D409H, L444P, R463C) are well known (Fig. 6.5). It has been reported that the mutation prevalence among various races is different. Mutations N370S, L444P, c.84–85insG, and IVS2 + 1G → A account for more than 95% of the mutated alleles in Ashkenazi Jewish patients, although they constitute less than 75% of the mutated alleles in non-Jews (Horowitz 1993). Among Japanese patients with Gaucher disease, neither mutation N370S nor c.84–85insG has not been identified (Ida et al. 1995) (Fig. 6.5). In the Portuguese Gaucher population, the N370S mutation accounts for 63% of the mutated alleles, and two other rare mutations, G377S and N396 T, are common (Amaral et al. 1999).

Interestingly, a nearly identical pseudogene is located 16 kb downstream from the functional gene. Sequencing of cDNA clones from patients with Gaucher disease showed complex alleles with several mutations. One complex allele had three point mutations, L444P, A456P, and V460 V, and has been designated as RecNciI. This complex allele could have been generated by a phenomenon where the pseudogene causes the transfer of mutations into the active gene via unequal, homologous recombination or gene conversion. The RecNciI allele is always associated with moderate to severe disease. The patients carrying L444P/RecNciI genotype always present with type 2 disease.

6.3.3 Genotype/Phenotype Correlation

The N370S mutation is highly linked with non-neuronopathic Gaucher disease (type 1) and has a very mild phenotypic expression. The existence of the N370S mutation implies that the patient has type 1 Gaucher disease. In addition, nearly 90% of the patients, carrying the N370S/N370S genotype, have a mild clinical course of the disease, and many of them are asymptomatic (Sibille et al. 1993). GBA protein carrying the N370S mutation is stable, has the same affinity toward the artificial substrate (4MU-β-glucoside) as the normal enzyme, but is not stable enough to catalyze its hydrolysis normally. Based on the characteristics of mutation prevalence in the Japanese population, incidence of patients with neuronopathic Gaucher disease is higher than that of Jewish patients, and Japanese type 1 disease tends to be more severe and progressive than the Jewish patients.

As shown in Fig. 6.6, the majority of Jewish patients are type 1. In contrast, approximately 50% of Japanese patients are classified into neuronopathic Gaucher disease (Tajima et al. 2009). Over 60% of Japanese patients have onset at less than 5 years of age. Among Japanese patients, 64% are splenectomized, and another 46% develop severe bone involvement. Over mean interval ranging from 4.9 to 6.9 years, mean relative height and weight, severity score index, and platelet counts all worsen to a highly significant degree (Ida et al. 1998) (Table 6.2).

6.4 Treatment

6.4.1 Enzyme Replacement Therapy (ERT)

Since ERT is safe and effective, it is the common treatment strategy for Gaucher disease and

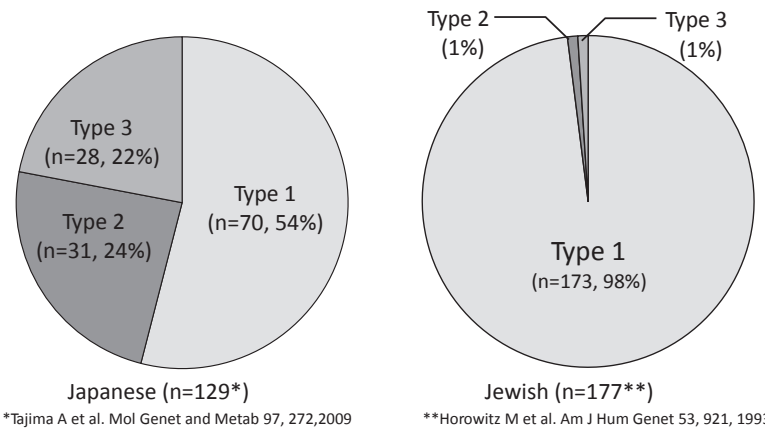


Fig. 6.6 Prevalence of clinical phenotypes in Gaucher disease Majority of Jewish patients are type 1 Gaucher disease (non-neuronopathic form). In contrast, approximately 50% of Japanese patients are classified into type 2 and type 3 diseases (neuronopathic form)

Table 6.2 Natural history of Japanese Gaucher disease type 1

	At baseline	At evaluation	Follow-up (year)	p-value#
Hemoglobin value (g/dl)	9.9±2.5	9.4±1.6	4.9	*
Platelet count ($\times 10^4/mm^3$)	11.5±7.9	7.7±5.5	4.9	**
Severity score index (SSI)	8.1±2.7	12.0±4.5	6.9	***
Body weight (SD)	–0.7±1.1	–1.2±0.8	5.0	**
Height (SD)	–1.9±1.0	–2.7±1.3	5.1	**

The values are expressed as mean values±SD, respectively
#In statistical analysis, paired t-tests were used to analyze changes in relative physical growth, and Wilcoxon signed-rank tests, to analyze changes in SSI and hematological measurements
*:p>0.05, **:p<0.01, ***:p<0.001

widely used. By reduction of the amount of accumulated glucocerebroside, hepatosplenomegaly, hematological abnormalities, and bone manifestations improve. The therapeutic enzyme is produced by DNA recombinant techniques using Chinese hamster ovary cells or genetically engineered human cultured skin fibroblasts. The terminal sugar on the glycosyl chains added on to the enzyme at the sites of glycosylation are modified to high-mannose (two N-acetylglucosamines with multiple mannose residues) molecule to target the enzyme to macrophages, via the macrophage mannose receptor. Enzyme therapy is administered by drip infusion every 2 weeks. Exogenous enzyme incorporates into monocytes/macrophages and is subsequently transported to lysosomes, ultimately degrading accumulated glucocerebroside.

One report of ERT between 2 and 5 years of treatment in 1028 adult patients with type 1 Gaucher disease demonstrated that ERT was effective for hematologic, visceral, and skeletal involvement (Weinreb et al. 2002). In another study, within 8 years of initiating ERT for 884 children with Gaucher disease type 1, most clinical parameters became normal or nearly normal (Andersson et al. 2008). However, neurological involvement does not respond to ERT since the administered enzyme does not cross the blood-brain barrier.

6.4.2 Human Stem Cell Transplantation (HSCT)

HSCT has the goal of replacing glucocerebrosidase activity in Gaucher patients. This is a consequence of the transplanted monocyte/macrophage system from donor cells expressing normal glucocerebrosidase. A small group of patients with type 3 disease (Norrbottnian type) has been successfully treated by HSCT (Ringden et al. 1995).

Interestingly, these patients had no further neurological deterioration posttreatment, perhaps due to the replacement of macrophages surrounding blood vessels in the central nervous system with healthy donor cells. The indications for HSCT are uncertain. HSCT costs are less than ERT and may prevent and arrest the neurological symptoms for type 3 patients. It is difficult to recommend HSCT for type 1 disease because of the 10% mortality rate posttransplantation and adverse long-term effects on growth and development.

6.4.3 Pharmacological Chaperone Therapy (PCT)

PCT is a new treatment strategy for Gaucher disease. The basic mechanism of PCT is to correct the folding and stabilize mutated GBA proteins, resulting in an increase in enzyme activity as well as increased concentration of enzyme (Parenti 2009). The mechanism of PCT is shown in Fig. 6.7. Orally administered chaperones enter the cell and bind to less stable, misfolded enzyme. The stabilized enzyme becomes properly folded and enters the lysosomes, where it continues to break down substrate. Since pharmacological chaperones are small molecules and designed to cross the blood-brain barrier, it is one of the candidates for the treatment of neuronopathic Gaucher disease that is not responsive to ERT. On the other hand, PCT is effective only for patients carrying specific mutations that affect enzyme stability. A clinical trial of PCT for neuronopathic Gaucher disease is underway (Narita et al. 2016).

6.4.4 Substrate Reduction Therapy (SRT)

SRT is an approach involving partial inhibition of glucosylceramide synthase, the enzyme that transfers glucose to ceramide to form glucosylceramide (glucocerebroside), to more evenly balance the rate of synthesis with the impaired rate of catabolism (Fig. 6.3). Since the SRT is an oral drug, patients do not have to visit a hospital every 2 weeks for administration by drip infusion. SRT for untreated adults with Gaucher disease type 1 resulted in significant improvement in spleen and liver volume, hemoglobin level, and platelet count. In addition, SRT maintained hematological and organ volume stability in adults with type

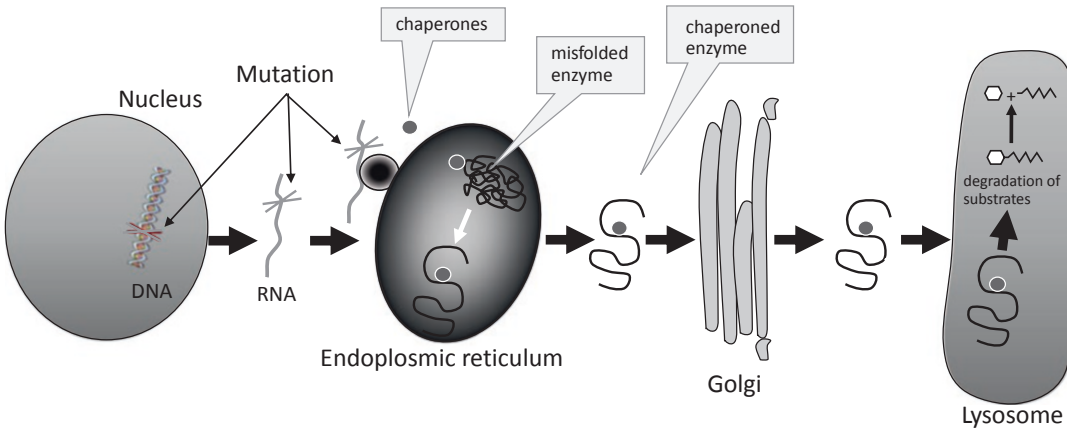


Fig. 6.7 Proposed mechanism of pharmacological chaperone therapy (PCT)
Chaperone binds the misfolded enzyme protein and changes the structure. It enables the enzyme to be stable and to go to Golgi complex. And the chaperoned enzyme enters the lysosome, and it degrades the accumulated substrate

1 Gaucher disease already controlled by ERT and could be useful as maintenance treatment (Cox et al. 2015). Major problems of SRT are adverse gastrointestinal effects and interactions with medications related to CYP2D6 metabolism (e.g., classes 1 and 3 antiarrhythmic drugs, selective serotonin reuptake inhibitors, tricyclic antidepressants, and so on).

End of Chapter Questions

1. Explain pathophysiology of Gaucher disease.
2. Explain the clinical manifestations of Gaucher disease.
3. Explain the treatment for Gaucher disease. And explain advantage and disadvantage of each treatment.

References

Amaral O, Lacerda L, Marcao A, Pinto E, Tamagnini G, Miranda MCS (1999) Homozygosity for two mild glucocerebrosidase mutations of probable Iberian origin. Clin Genet 56:100–102

Andersson H, Kaplan P, Kacena K, Yee J (2008) Eight-year clinical outcomes of long-term enzyme replacement therapy for 884 children with Gaucher disease type 1. Pediatrics 122:1182–1190

Cox TM, Drelichman G, Cravo R, Balwani M, Burrow TA, Martins AM, Lukina E, Rosenbloom B, Ross L, Angell J, Puga AC (2015) Eliglustat compared with imiglucerase in patients with Gaucher's disease type 1 stabilized on enzyme replacement therapy: a phase 3, randomized, open-label, non-inferiority trial. Lancet 385:2355–2362

Horowitz M, Tzuri G, Eyal N, Berebi A, Kolodony EH, Brady RO, Barton NW, Abrahamov A, Zimran A (1993) Prevalence of nine mutations among Jewish and non-Jewish Gaucher disease patients. Am J Hum Genet 53:921–930

Ida H, Iwasawa K, Kawame H, Rennert OM, Maekawa K, Eto Y (1995) Characteristics of gene mutations among 32 unrelated Japanese Gaucher disease patients: absence of the common Jewish 84GG and 1226G mutations. Hum Genet 95:717–720

Ida H, Rennert OM, Ito T, Maekawa K, Eto Y (1998) Type 1 Gaucher disease: phenotypic expression and natural history in Japanese patients. Blood Cells, Mol Dis 24:73–81

Narita A, Higaki K, Maegaki Y, Ohno K, Suzuki Y (2016) Ambroxol chaperone therapy for neuronopathic Gaucher disease: a pilot study. Ann Clin Translat Neurol 3:200–215

Parenti G (2009) Treating lysosomal storage diseases with pharmacological chaperones: from concept to clinics. EMBO Mol Med 1:268–279

Ringden O, Groth CG, Erikson A, Granqvist S, Mansson JE, Sparrelid E (1995) Ten years's experience of bone marrow transplantation for Gaucher disease. Transplantation 59:864–870

Sibille A, Eng CM, Kim SJ, Pastores G, Grabowski GA (1993) Phenotype/genotype correlations in Gaucher disease type 1: clinical and therapeutic implications. Am J Hum Genet 52:1094–1101

Tajima A, Yokoi T, Ariga M, Ito T, Kaneshiro E, Eto Y, Ida H (2009) Clinical and genetic study of Japanese patients with type 3 Gaucher disease. Mol Genet Metab 97:272–277

Weinreb NJ, Charrow J, Andersson HC, Laplan P, Kolodony EH, Mistry P, Pastores G, Rosenbloom BE, Scott CR, Wappner RS, Zimran A (2002) Effectiveness of ERT in 1028 patients with type 1 Gaucher disease after 2 to 5 years treatment. Am J Med 113:112–119