

Gaucher disease: a lysosomal neurodegenerative disorder

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Abstract. – Gaucher disease is a multisystemic disorder that affects men and woman in equal numbers and occurs in all ethnic groups at any age with racial variations and an estimated worldwide incidence of 1/75 000. It is caused by a genetic deficient activity of the lysosomal enzyme glucocerebrosidase due to mutations in the β -glucocerebrosidase gene, and resulting in lack of glucocerebroside degradation. The subsequent accumulation of glucocerebroside in lysosomes of tissue macrophages primarily in the liver, bone marrow and spleen, causes damage in haematological, skeletal and nervous systems. The clinical manifestations show a high degree of variability with symptoms that varies according to organs involved. In many cases, these disorders do not correlate with mutations in the β -glucocerebrosidase gene. Although several mutations have been identified as responsible for the deficient activity of glucocerebrosidase, mechanisms by which this enzymatic defect leads to Gaucher disease remain poorly understood. Recent reports indicate the implication of complex mechanisms, including enzyme deficiency, substrate accumulation, unfolded protein response, and macrophage activation. Further elucidating these mechanisms will advance understanding of Gaucher disease and related disorders.

Key Words:

Gaucher disease, Glucocerebrosidase, Macrophage, Glucocerebroside, Unfolded proteins, Genotype, Clinical manifestations.

Introduction

Gaucher disease is a multisystemic disorder that results from the accumulation of undegraded glucocerebroside in lysosomes of tissue macrophages due to deficiency of the lysosomal enzyme glucocerebrosidase caused by mutations in β -glucocerebrosidase gene. Macrophages engorged with glucocerebroside primarily infiltrate liver, spleen and bone marrow, leading to skeletal, haematological and visceral manifestations. The clinical manifes-

tations commonly associated with Gaucher disease include hepatosplenomegaly, anemia, thrombocytopenia, bone disease and, in some patients, neurological disorders. Gaucher disease has classically been categorized into three subtypes that are distinguished by the presence or absence of neurological manifestations¹. Type 1 is the non-neuronopathic form and the most frequent, accounting for 95% of Gaucher disease cases². It occurs at any age, but progresses rapidly if the onset occurs prior to adulthood without central nervous system (CNS) involvement³. Types 2 and 3 are both neuronopathic forms affecting the CNS, but manifesting at different degrees of neurological deterioration. Type 2 is the acute neuronopathic form, it manifests with devastating neurological deterioration. Patients with type 2 generally present with signs either prenatal or during infancy, and usually die before the age of 3 years². Type 3 is the chronic neuronopathic form and has more attenuated neurological features with pathognomic supranuclear horizontal gaze palsy, accompanied by visceral manifestations. This review summarizes the current knowledge regarding the incidence, cause and mechanisms interplaying in Gaucher disease, and presents an overview of disorders associated with this disease.

Incidence

Gaucher disease affects men and woman equally and can occur at any age in any human population⁴. It occurs in approximately 1:20 000 to 1:200 000 live births in the general population, but is more prevalent in Ashkenazi Jewish population with an estimated incidence rate of 1:450 live births². Type 1 is the most prevalent disease form and affects this population with a greater incidence estimated at 1:12⁵. In non-Jewish population, the incidence of type 1 is estimated at 1:50 000 to 1:75 000 in North America, Europe and Australia^{4,6}, and at 1:20 000 or a prevalence of 1:40 000 in United State. Type 2 has an estimated incidence of 1:150 000⁷. This type is not

prevalent comprising about 1% of patients compared with 5% for type 3. The estimated incidence of type 3 is 1:200 000, but the prevalence is considerably greater than type 2. This type is panethnic, but with well-studied clusters in Northern Europe, Egypt and East Asia⁸.

The prevalence of mutations responsible for Gaucher disease varies with ethnicity, and a wide variation in their distribution is seen in different populations. The four most common mutations identified as N370S, IVS2 (+1), 84GG and L444P have significant frequencies in the Ashkenazi Jewish population, accounting for 98% of disease in this population⁹ and approximately 50%-60% in non-Jewish population¹⁰. A high prevalence of mutations in N370S and 84GG is found in Ashkenazi Jewish population, whereas mutations in N370S alone are prevalent in North America, Europe, and Israel population¹¹. In non-Jewish populations, L444P and N370S are the most common mutations, but the N370S allele is frequent in Caucasian population, and absent in Asian population in which the L444P allele represents the most common^{12,13}.

Spectrum of Disorders Related to Gaucher Disease

Since the discovery of Gaucher disease by Philippe Gaucher in 1882, the spectrum of clinical variation in Gaucher disease has continued to evolve. Today, the Gaucher phenotype includes various disorders in skeletal, haematological and visceral systems.

Skeletal disorders: The spectrum of typical skeletal disorders include osteopenia, osteoporosis, focal lytic or sclerotic lesions, pathological fractures, growth retardation, bone pain, painful or bone crisis, decreased mineralization, osteonecrosis or vascular necrosis, cortical and medullary infarcts. These disorders contribute much to the morbidity and disability associated with Gaucher disease and are most commonly seen in types 1 and 3. Positive correlation between decreased bone mineral density and disease severity has been found in patients with the N370S/84GG genotype¹⁴. Although events leading to skeletal disorders in Gaucher disease are unknown, following mechanisms have been proposed to explain a part of the symptoms: altered bone formation and resorption, as well as bone marrow infiltration and increased intra-osseous pressure due to the infiltration leading to vascular

occlusion. Indeed, the replacement of marrow by engorged macrophages can lead to expansion of the medullary cavity with thinning of the cortex and endosteal scalloping and consequent osteopenia. Moreover, the medullary expansion leads to a failure of remodeling in the distal femurs and the proximal tibia deformity. The lack of blood supply and the consequent bone death are responsible for osteonecrosis occurrence. The severity of bone disorders depends on the extent of medullary cavity replacement.

Haematological disorders: Abnormalities in the haematological system are very frequent in Gaucher disease with anaemia and thrombocytopenia as the most common and early signs. Splenomegaly associated with hypersplenism and depressed haematopoiesis due to engorged macrophages infiltration into bone marrow are considered as the primary causes of thrombocytopenia; anaemia can be multifactorial and related to hypersplenism, pancytopenia, iron deficiency, abnormalities in bone metabolism and transport, vitamin B12 deficiency and autoimmune haemolytic anaemia. Other haematological disorders include both polyclonal and monoclonal gammopathies with an increased risk of multiple myeloma. Immune dysregulation within the bone marrow microenvironment, leading to increased release of inflammatory cytokines, is believed to be the causative factor for this haematological malignancy or cancer. Symptoms of the haematological disorders include pallor, fatigue, easy bruising, frequent nosebleeds, exertional dyspnoea, and palpitation.

Visceral disorders: liver and spleen are the most common viscera affected in Gaucher disease. Engorged macrophages accumulate in the Kupfer cells of the liver and cause liver enlargement or hepatomegaly. The progression of hepatomegaly is nearly linear. Cirrhosis and fibrosis can develop, although portal hypertension is rare.

The accumulation of engorged macrophages within spleen leads to spleen enlargement or splenomegaly. In type 1 disease form, spleen volume is typically 5-15 times normal but in some cases it can significantly increase and exceed 50 times normal. Massive splenomegaly can result in many complications including fibrotic scarring, risk of rupture, splenic infarctus, nodules, and malignancies. An uncommon disorder is pulmonary involvement which more

frequently is encountered in type 1 patients who undergone splenectomy and those with type 3¹⁵. The lung disorders likely are secondary to direct infiltration by engorged macrophages into the interstitial spaces, alveolar spaces capillaries¹⁶. They can also be indirect causes secondary to hepatopulmonary syndrome related to the liver disorders associated neurological manifestations. Fibrosis and pulmonary hypertension are rare. In general, symptomatic pulmonary involvement is encountered in patients with more striking visceral and skeletal disorders. Many other organs including heart and kidney have involved. While renal involvement is often asymptomatic, cardiac involvement presents with serious manifestations such as pericarditis, valvular trouble associated with homozygosity for the D409H allele¹⁷, and myocardiopathy due to infiltration of myocardium¹⁸.

Neurological disorders: Peripheral neuropathy and Parkinson diseases occur in type 1; and both type 2 and 3 develop CNS disorders. A panel of CNS disorders, including dementia, seizure, myoclonus, generalized epilepsy, spasticity, ataxia¹⁹, uveitis, vitreous body lesions, retinopathy, and conjunctivitis^{20,21}, has been associated with type 3. Recently, myoclonic epilepsy has been added in the phenotypic spectrum of neuropathic Gaucher disease. While all probands had the classic type 3 horizontal gaze abnormality, the genotypes identified differed from those typically associated this type and included three alleles N188S, V39rL, and G377S. Both the variability in clinical phenotype and the lack of uniform genotype suggest the contribution of other modifiers to this rare phenotype.

Genetic Causes

Gaucher disease is caused by mutations in the β -glucocerebrosidase gene. This gene is localized on the paternally inherited chromosome 1q22 and consists of 10 introns and 11 exons²². Today, more than 300 mutations have been identified as the cause of Gaucher disease, and the most common mutations seen in 98% of disease alleles are, N370S, L444P, 84GG, and IVS2. Of these four mutations, N370S and L444P have predictive value on clinical outcomes²³. The presence of at least one N370S allele prevents the development of neurological manifestations and confers type 1 disease, whereas the presence of the L444P allele is highly associated with CNS in-

volvement. Moreover, homozygosity for the N370S allele predicts less severe manifestations of disease in adults, while heterozygosity with one copy of N370S and a second copy being L444P, 84GG or IVS2 (+1) predicts more severe neurological manifestations. Typically, adult patients with genotype data showing homozygous for L444P mutation have the type 3-neuronopathic diseases. This genotype indicates that the patient is at risk of severe disease, and fetuses with two null alleles are non-viable^{24,25}. However, there is still significant phenotypic heterogeneity with the same genotype, since phenotype and disease severity may vary among patients carrying the same mutations²⁶ and among siblings²⁷. Accordingly, many affected individuals with the N370S/N370S homozygous genotype may remain asymptomatic through life, while some patients with the same N370S/N370S homozygous genotype present with severe symptomatic disease, and a type 1 patient with the N370S/L444P genotype may have mild symptoms. This implies that beside mutations in the glucocerebrosidase gene, other factors such as genetic modifiers or environmental factors are important in the manifestation of the disease. Modifiers could include genes involved in other steps of the metabolic pathway, or post-transcription modifications affecting the trafficking, binding or degradation of glucocerebrosidase.

Deficiency of the Lysosomal Enzyme Glucocerebrosidase

Human glucocerebrosidase is a membrane-associated lysosomal glycoprotein that is encoded by a single locus at chromosome 1q21²⁸. It has 497 amino acids and N-glycosylation at four or five different sites that are critical for its catalytic activity and half-life²⁹. In the lysosome, glucocerebrosidase catalyzes a two-step reaction that requires glycosylation of its active site by the substrate, followed by the release of β -glucose by deglycosylation³⁰. Its catalytic activity is enhanced by its physical interaction with saposin C. The majority of glucocerebrosidase mutations are spread through the domains of the protein and lead to the synthesis of glucocerebrosidase with decreased catalytic function and/or stability³¹; while few mutations that are associated with a severe phenotype, occur near the catalytic site and lead to the synthesis of glucocerebrosidase with decreased stability. Accordingly, the mutation N370S that is associated with non severe phenotype, has been suggested to flank the active

site, and alter interaction with saposin C and anionic phospholipid membranes³², whereas the mutation L444P, which is associated with a severe phenotype, is located at the hydrophobic core of the protein, and is predicted to produce unstable glucocerebrosidase³³. However, the relationship between the position of mutations in the 3D structure and the severity of the disease is not clear³⁴. In contrast, it is clearly assumed that disease severity depends on residual glucocerebrosidase activity.

Ungraded Substrate Accumulation

There is no doubt that the abnormal glucocerebrosidase accumulation is one of the primary pathogenic factors in Gaucher disease. Glucocerebrosidase is hydrolyzed to ceramide and glucose by glucocerebrosidase. In Gaucher disease, the activity of glucocerebrosidase is disrupted and glucocerebrosidase accumulates within lysosomes. It is not clear whether the cellular pathology is related to the lysosome dysfunction, or if glucocerebrosidase escapes from the lysosomes and directly interacts with other organelles. Evidence for lysosomal dysfunction comes from the finding of other lysosomal enzymes in patients with Gaucher disease³⁵ and abnormal autophagosome formation³⁶. Nevertheless, the notion of direct interaction with organelles is not totally excluded, since glucocerebrosidase is elevated in plasma and accumulates in various organs of patients. Glucosylsphingosine, another substrate of glucocerebrosidase, also accumulates in the lysosome, and

is elevated in plasma and organs of patients^{37,38}. This lipid has been suggested to have a role in the pathogenesis of both bone disease³⁹ and haematological malignancies in Gaucher disease⁴⁰ and also to contribute to the CNS involvement in patients with neuronopathic forms³⁸. Since glucosylsphingosine is known to be a highly cytotoxic compound, it is considered to have far-reaching signaling effects and may be responsible for cell death⁴¹.

Unfolded Protein Response

Unfolded proteins have been involved in the pathogenesis of Parkinson⁴². Clinical, genetic and neuropathologic observations have revealed similarities between a heterozygous variant for a glucocerebrosidase mutation and Parkinson disease, suggesting a role for unfolded proteins in the phenotype associated with Gaucher disease^{43,44}.

The majority of glucocerebrosidase mutations identified in patients with Gaucher disease are missense³¹. After transcription, the nascent glucocerebrosidase protein undergoes folding in the endoplasmic reticulum (ER). When correctly folded it shuttles to the Golgi compartment for further modifications and finally it traffics to the lysosomes to hydrolyze its substrates, glucocerebrosidase and glucosylsphingosine. However, some mutations affect the catalytic site causing a deficiency in glucocerebrosidase and leading to accumulation of both glucocerebrosidase and glucosylsphingosine (Figure 1). Moreover, many other mutations result in conformational changes that cause misfolding

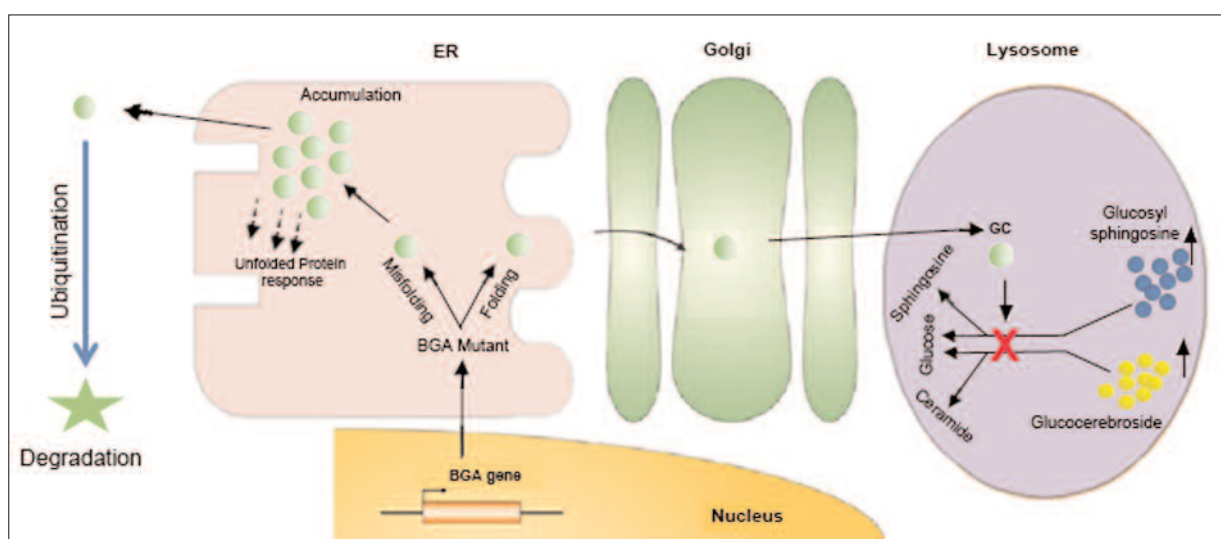


Figure 1. Simultaneous accumulation of unfolded proteins and glucocerebrosidase in the reticuloendothelial system. GBA: β-glucocerebrosidase; GC: glucocerebrosidase. Please, refer to the text for details.

within the ER. Mis-folded proteins are retained in the ER or transported to the cytoplasm where they are ubiquitinated and undergo proteasomal degradation. Mis-folded proteins accumulated within the ER, activate a sequence of cellular responses known as the unfolded protein response (Figure 1). Such phenomena have been reported in both patient with Gaucher disease and heterozygote carrier⁴⁵, and has been implicated in the pathogenesis of Parkinson disease.

Macrophage Activation

More than a century ago, Gaucher disease was described by the identification of lipid engorged macrophages, leading to the classification of Gaucher disease as a macrophage disorder. However, the simple presence of storage material within these infiltrating cells cannot provide a complete explanation of causation; and the current knowledge make this classification too simplistic. Indeed, engorged macrophages are not inert cells; they are metabolically active and secrete

various proteins involved in inflammation, chemotaxis, differentiation and hypergammaglobulinaemia (Figure 2). Briefly, macrophages activated by excess of glucocerebroside secrete inflammatory cytokines, which cause enlargement of the spleen and liver, destruction of bone, abnormalities in the lung, anaemia, thrombocytopenia and leukopenia^{46,47}. These macrophages also secrete MCP1 and CXCL/IL-8 that cause the recruitment of blood monocytes into the visceral organs, where they mature into macrophages, which in turn activate the release of IFN- γ , IL-4 and IL-6. The cytokines IFN- γ and IL-4 activate Th-1 and Th-2 cell-mediated responses, whereas IL-6 facilitates the development of follicular T cells (Tfh). These responses lead to the formation and activation of the germinal center that triggers B-cell differentiation and immunoglobulin (IgG, IgA and IgM) production, and consequently hypergammaglobulinemia. Such mechanism has been proposed in the pathogenesis of malignancy in Gaucher disease⁴⁸.

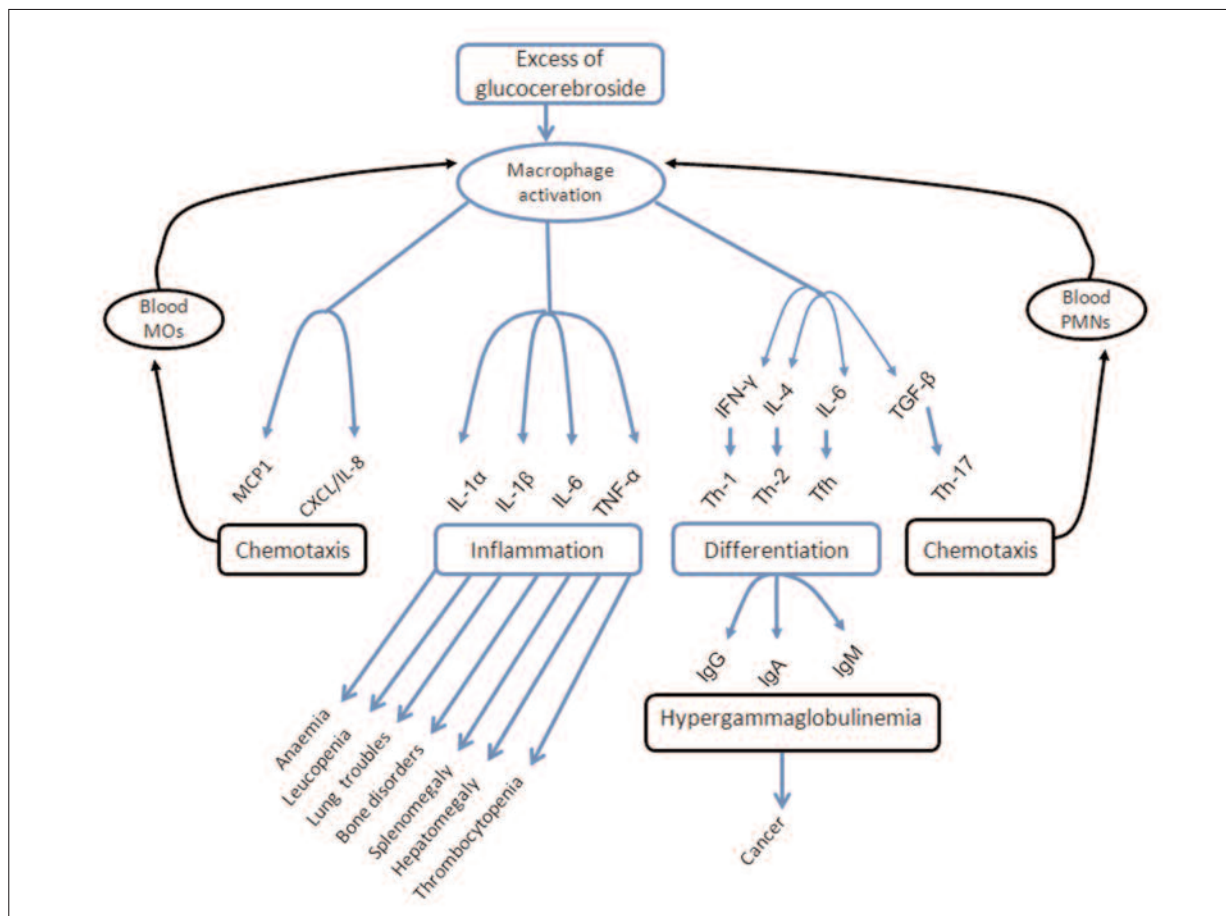


Figure 2. Macrophage response to glucocerebroside accumulation. Please, refer to the text for details.

Enzyme Replacement Therapy

Vegetable derived recombinant enzyme: among the therapeutic approaches available for gaucher disease is enzyme replacement therapy is important. However the source of recombinant acid β -glucocerebrosidase and its availability has a significant impact on this approach. Genzyme's pioneer product imiglucerase, commercialized as Cerezyme, is produced in CHO cells whereas Shire's velaglucerase alfa, sold as Vpriv, is made in a human cell line. However, these methods of recombinant enzyme production are susceptible for contamination by different types of virus that can infect mammalian cell. For example, Cerezyme's contamination by in 2009 by vesivirus2117 caused a shutdown of its production for a longtime⁴⁹. To circumvent these problems, investigators now produced β -glucocerebrosidase, as Taliglucerase- α in carrot root cells, which does not suffer from several of the drawbacks associated with recombinant protein production in mammalian or bacterial cells. Taliglucerase- α is now approved in USA and other countries and is found to be effective in treating Gaucher disease⁵⁰.

Conclusions

The study of the aetiology and pathophysiology of Gaucher disease may take on greater importance inside the research fields of neurodegenerative diseases and malignancies. Particularly, the association of Gaucher disease with Parkinson disease and multiple myeloma is suggestive of a possible role of the aberrant glycosphingolipid metabolism in the pathogenesis of more common disorders. Moreover, recent insights into the bone marrow environment and the unfolded protein response provide promising areas for further exploration. It may be anticipated that further advance in the understanding of these mechanisms will continue to provide new and important information for a better understanding of more common disorders.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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