Glucocerebrosidase Deficiency: A Leading Cause of Acute Neurological Disorders in Humans

Rida Nasir Butt and Sikander Ali
Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan

ABSTRACT

Glucocerebroside is accumulated within the lysosomes due to the deficiency of GCase. Mutation in GBA 1 gene is responsible for the insufficient quantity of GCase. Particularly, GD type 2 is neuronopathic and leads to infantile death. The mis- sense mutation in L444P or N370S heteroallelism of GBA 1 gene at chromosome 1q21 leads to inhibit the production of GCase. Insufficient GCase is guilty for the deposition of glucocerebroside and does not permit the degradation of glucocerebroside into ceramide and glucose. It results in impaired cell signaling, neuronal death affecting CNS, hydrops fetalis, brainstem non- functioning, epilepsy, convulsions, dementia and mental retardation. The neurological dysfunctioning in infants lead them to death before they reach an age of 2 years. For the treatment of this disease, chaperone therapy is recommended as the chaperone are able to cross the bloods brain barrier and can enter into the brain cells keeping the infants from neurological deterioration.

Keywords: GBA 1 gene, GCase enzyme, glucocereboside, neurological deterioration.

INTRODUCTION

An autosomal recessive metabolic disease caused by the deficiency of lysosomal glucocerebrosidase enzyme (GCase) is called Gaucher disease. Due to mutation in GBA 1 gene at chromosome 1q21, GCase leads to accumulation of glucocerebroside and does not allow the transformation of glucocerebroside into ceramide and glucose. It is because the mutation in GBA 1 gene stops the production of enzyme GCase responsible for this degradation. [8][16] Deposition of glucocerebroside within lysosomes of macrophages give birth to Gaucher cells, whose cytoplasm looks like a wrinkled tissue paper and have displaced nucleus. They have distorted lysosomes in them. These distorted lysosomes were observed by an electron microscopy. [33] Gaucher disease (GD) shows three different types on the basis of neurological disorder. (Table 1) Type 1 is neuronopathic whereas type 2 and 3 are neuronopathic and are differentiated on the basis of their level of progression and age of onset. They cannot be distinguished depending on the amount of lipid stored and enzyme activity. GD type 2 is the rarest and the most severe neuronopathic disorder affecting infants only. [37] Usually the infant suffering from this disease die before reaching to the age of 2 years. [38]

In 1882, Philippe Gaucher hypothesized a disease (Gaucher 1882) whose biochemical basis was elucidated by Roscoe Brady’s group at National institute of health in 1965. [7] The disease was named as Gaucher disease after a scientist who hypothesized it. The molecular basis of it was discovered in 1980s which revealed its cause. The gene mutations in GCase were found to be responsible for this disorder. [4][20] Experiments were made on mouse in order to study the effects of disease. With the development of tactics to make mouse models of human disease, there were great hopes that the formation of glucocerebrosidase deficient mice would help in understanding of both the pathophysiology of Gaucher disease and the phenotypic outcomes of specific human mutations. Studies in mice led to completely unexpected research directions and to significant medical insights, but till now there are still no feasible mouse models that are comparable to much common human phenotype. The first mouse model was created with a null allele and resulted in a neonatal fatal phenotype. [40]

*Address for correspondence:
alishbiotech@yahoo.com
Table 1. Classification of Gaucher Disease

<table>
<thead>
<tr>
<th>Features</th>
<th>Type-1 (Non-neurono-pathic)</th>
<th>Type-2 (Neurono-pathic)</th>
<th>Type-3 Neurono-pathic (juvenile)</th>
<th>Bibliography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Involvement of central nervous system(CNS)</td>
<td>No CNS problem</td>
<td>Brainstem abnormalities in early age. Acute neurological decline. Spasticity and Strabismus</td>
<td>Later onset of CNS problem</td>
<td>10, 38</td>
</tr>
<tr>
<td>Striking rate</td>
<td>More common in Ashkenazi Jewish population (1 in 450) General population (1 in 100000)</td>
<td>1 in 100000 live births</td>
<td>1 in 50000 live births</td>
<td>10</td>
</tr>
<tr>
<td>Survival</td>
<td>Varies</td>
<td>&lt; 2 years of age</td>
<td>From childhood to adult</td>
<td>38</td>
</tr>
<tr>
<td>Therapy</td>
<td>Enzyme replacement therapy. Substrate reduction therapy</td>
<td>No specific therapy. Palliative</td>
<td>Enzyme replacement therapy. Bone marrow transplantation</td>
<td>38</td>
</tr>
</tbody>
</table>

Homozygous mice died within hours of birth. These newborn mice led to the appreciation of a similar human phenotype, infants that expire due to Gaucher disease either in utero, or at birth. Based upon the population studies in the Netherlands, the perinatal lethal form of GD type 2 was actually found to be more common than the classic type 2 GD, although these cases often go undiagnosed. GD type 2 in particular was first identified as a distinct phenotype in 1927.

**Figure 1. Role of glucocerebrosidase enzyme (GCase) The enzyme degrades the glucocerebrosidase and glucosylsphingosine into ceramide+glucose and sphingosine+glucose, respectively.**

Macromolecules are degraded and recycled by the membrane bounded organelles known as lysosomes. Specific lysosomal hydrolases are responsible for degrading glucosphingolipids like those of glucosylsphingosine and glucosylceramide within the lumens of the lysosomes. GCase breaks the glucocerebrosidase into glucose and ceramide. This breakdown of glycolipid to its simpler units is necessarily required by the different tissues and organs to perform their proper function. Ceramide and glucose are the smaller units to which glucocerebrosidase is converted by the GCase in lumen of the lysosome. GCase is also involved in degrading glucosylsphingosine into glucose and sphingosine units.

**PATHOPHYSIOLOGY**

**Mutation: Cause of Gcase Deficiency**

Mutation in GBA (glucosidase beta acid) 1 gene results in improper activity of GCase or inhibits its production. The deficiency of GCase does not allow the degradation of glucocerebrosidase into its component smaller units (glucose and ceramide) and results in accumulation of glucocerebrosidase in the lysosome. The gene that encodes for GCase is located on chromosome 1q21 and constitutes 11 exons. More than 200 mutations have been recognized for GD. These mutations may be mis-sense, splice-site, frame-shift, deletion, and insertion or recombinant alleles. The most frequent mutations are N370S, 84GG, L444P and IVS2. N370S heteroallelism or mis-sense mutation in L444P.
usually results in GD type 2. [8] L444P/L444P point mutations or recombinant alleles are linked to type 2 GD. The existence of two recombinant alleles seem to predispose to hydrops fetalis and prenatal lethality. Homozygosities for c.533del C, R131L, S196P, H311R or G202R mutations are correlated with GD type 2. These mutations may result in improper folding of protein and no enzyme is produced. [35]

**Effected Pathway**

GCase, a lysosomal enzyme, is synthesized on polyribosomes bounded to endoplasmic reticulum (ER). GCase has two size leaders: a 38 amino acids leader for first AUG and a 19 amino acids leader for the second AUG. AUG, a start codon, serves as an initiator methionine. [29] Its passage into the ER is complemented by leader peptide cleavage and N-linked glycosylation on 4 asparagine residues [Asn- 19, Asn- 59, Asn- 146 and Asn 270]. [15] The improper folding does not allow the enzyme to enter the Golgi network and then to lysosomes. The misfolded molecules of GCase are kept in the ER. and are not delivered to the lysosomes. [42] In result, GCase becomes deficit in the lysosomes and accumulates the substrate glucocerebroside.

**Lipid Deposition**

Figure 2. The pathway involving glucocerebrosidase enzyme. Glucocerebrosidase enzyme converts glucocerebroside (glucosylceramide) into its components glucose and ceramide. Deacetylation of glucocerebroside forms glucosylsphingosine. Ceramidase converts ceramide into sphingosine, whose glycosylation also produces glucosylsphingosine. [38]

The insufficient GCase leads to deposit glucocerebroside in lysosomes of macrophages giving rise to Gaucher cells. These cells have distorted lysosomes and displaced nucleus. [12] Along with the glucocerebroside, glucosylsphingosine is also deposited. Deacetylation of glucocerebroside or glycosylation of shingosine forms glucosylsphingosine. [12]

Deposition of the glycolipids does not let their breakdown into smaller units. The component little units glucose, sphingosine and ceramide are necessarily required by the different cell organelles, tissues and organs for their proper functioning. This glycolipid storage leads to the mal-functioning of various parts of the body, especially brain. This accumulation of macromolecules in the cells cause impaired cell signaling, release of Ca²⁺ from endoplasmic reticulum, [30] weakened autophagocytosis and endocytosis, [36] and inhibition of cytokinesis. [23] Deficient enzyme resulting in lipid deposition in Gaucher cells aggregate α-synuclein. Apoptosis occur and the contents especially elevated α-synuclein is released with the bursting of macrophages. This over increased α-synuclein is either directly infected by the neurons or indirectly with the enhanced exosome secretion. It is toxic to human neurons so, when it is infected by the neurons, it causes damage to substantia nigra. [41] (Figure 3) Over expressed level of a small protein, a- synuclein, is toxic and is linked with an increased rate of neuronal cell death. Substantial loss of different types of neurons, involving the dopaminergic cells of the substantia nigra are the main focus of recent symptomatic therapies. [11] Increased level of glucosylsphingosine affects the central nervous system (CNS) and leads to neuronal death. [27] Mice were used as an experimental model for the study of GD type 2. Tissues obtained from GD type 2 patients and mice showed that the accumulation started in Gestation period. [28]
ASSOCIATION OF PARKINSON’S DISEASE WITH GD TYPE 2

Parkinson’s disease is principally due to a gradual degeneration of dopaminergic neurons in the substantia nigra and other monoaminergic cell groups of the brain. Symptoms emerge when between 50-70% of the dopaminergic neurons are lost. There are different suppositions about how to explain the link between Parkinson disease and GD type 2. One of them says that mutations in GBA gene can produce an unusual and deviant protein with function gain either hetero or homozygous and enable α-synuclein aggregation. This effect can induce neuronal poisonousness and toxicity. Wild-type α-synuclein can translocate into lysosomes for the degradation of glycolipids by the chaperone mediated autophagy pathway. The stock of misfolded mutated glucocerebrosidase protein in the endoplasmic reticulum cause a state of trauma and stress. It also decreases the activity of ubiquitin ligase, linked with the occurrence of early onset of Parkinson disease. This stress can initiate the mechanisms of neuronal apoptosis in the substantia nigra, and hence the onset of GD type 2.

SYMPTOMS OF GD TYPE 2

The deficit enzyme causing neurological dysfunctioning in infants lead them to death. The symptoms reported for the GD type 2 are the following: hepatomegaly, splenomegaly, anemia, thrombocytopenia, poor fetal movement, non-immune hydrops fetalis with or without the excessive storage of fluid in abdominal cavity and arthrogryposis. Hydrops fetalis increases the chance of fetal death. Those who survive to delivery, die in first week of their life. The absence of hydrops rapidly worsens the neurological function leading to death within 3 months. At birth, ichthyosis (collodion like skin) may be observed in such patients. Congenital ichthyosis leads to hyper-proliferation along with the hypertelorism, low set ears, everted lips, microstomia and anteverted nares. Cortical and brainstem dysfunctioning results in neonatal distress, microcephaly and central apnea. Death occurs in first month of age due to neurological involvement. The most frequent initial bulbar signs include strabismus and hyperextension of neck which worsens upon stimulation. Other neurological signs include epilepsy, extrapyramidal tract involvement, trismus, stridor, dementia, convulsions, mental retardation and ocular muscle apraxia. Due to central apnea, death usually occurs by 2 years of age.

STRIKING RATE
It is very rare infantile disease with no ethnicity. 1 in 100000 infants get affected by GD type 2.\textsuperscript{[10]}

(Figure 4)It had only 11 published cases till 2011\textsuperscript{[1]} GD type 2 neuronopathic variants represent <1% of patients in Europe, West America and Israel. It has more frequency in Middle East, Indian subcontinent, China, Korea and Japan.\textsuperscript{[9][19]} The most frequent mutation in L444P was observed in non-Ashkenazi Jewish population exhibiting neurological disorder. Surprisingly, Japanese populations have L444P allele either in heteroallelic or homoallelic forms leading to the neuronopathic disease.\textsuperscript{[13]} The gene frequency of N370S alleles with mutation in Ashkenazi population is 1.03/1000. In non-Jewish patients its frequency is comparatively low: 1/21500 in Portugal, 1/86000 in Netherland, 1/40247 in Italy and 1.13/100000 in Czech Republic. This indicates that GD type 2 neuronopathic disorder is more common in Ashkenazi Jewish population.\textsuperscript{[8]}

CONSEQUENCES OF LACKING GCCase OBSERVED IN SOME PATIENTS

Case 1

The paucity of GCCase piles up the glycolipids and causes a neuropathy with destruction of neurons within the CNS, leading to severe brainstem malfunctioning or progressive neurological depreciation. A case of GD type 2 was reported in a female Mexican infant. She was born at 34 weeks gestation and died at 2 days of age. The observed symptoms in her were collodion membrane, arthrogryposis, extensive loss and destruction of neurons in brain and spinal cord. GCCase activity was absent in all tissues and glucocerebroside was found to be accumulated in microglia and nerve cells of the brain.\textsuperscript{[14]}

Case 2

Another case with perinatal lethal GD was reported in an Afghan family having acute fetal hypokinesia with several joint contractures and hydrops fetalis with bilateral hydrothorax. The infant was born at 33 weeks gestation period and survived for less than an hour after delivery.\textsuperscript{[32][40]}

TREATMENT

The expected survival is <2 years because GD type 2 has no disease specific therapy. For the treatment of GD, recombinant GCCase (imi-glucerase) is used but this enzyme replacement therapy has proved not be effective in case of type 2.\textsuperscript{[31]} It is because recombinant GCCase cannot cross the blood brain barrier. As it is not possible for the recombinant enzyme to pass the blood brain barrier, severe neurological disorder central apnea will lead the patient to death.\textsuperscript{[38]} Substrate reduction therapy also failed to treat GD type 2. A chaperone therapy has emerged to treat GD type 2 in which chaperones can diffuse into the brain cells and stabilizes the level of enzyme GCCase.\textsuperscript{[22]}

RECOMMENDATIONS

For GD type 2, pharmacological chaperones are recommended which can combine to the misfolded proteins in ER, aid their folding and transfer them to their target (lysosome). In lysosomes, GCCase must have their activity to degrade the deposited glucocerebroside into glucose and ceramide. As chaperones are minute molecules, so they can pass through the blood brain barrier and enter into the brain cells. This is how neurological disorder can be prevented. Mostly imino-sugars are used. Imino-sugars are the molecules having nitrogen instead of oxygen atom in the glucose ring. Imino-sugar chaperones are mostly used because they bear the structural resemblance with glycoside of glucosylceramide. It restores the enzyme activity and allows the degradation of deposited glycolipids.\textsuperscript{[3]} Chaperone therapy can keep the infants from neurological deterioration and can save their worthy lives.

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CONFLICT OF INTERESTS

None

REFERENCES


