INTRODUCTION

1 INTRODUCTION

1.1 Lysosomal storage disorder (LSD)

The most common cause of inherited disease is aninborn error of metabolism (Burton, 1998), of which lysosomal storage diseases (LSDs) are a significant subgroup (Platt and Walkley, 2004; Fuller et al., 2006; Ballabio and Gieselmann, 2009). LSDs are rare inherited metabolic disorderswith the combined incidence of approximately 1:5,000 live births (Fuller et al., 2006), but the true figure is likely to be higherwhen undiagnosed or misdiagnosed cases are accounted. Itconsists of a heterogeneous group of almost 50 disorders that are caused by genetic defects in a lysosomal acid hydrolase, receptor, activator protein, membrane protein, or transporter, causing lysosomal accumulation of substrates that are specific to each disorder (Wang et al., 2011). Most of the LSDs result from deficiency of acidic hydrolase enzymes (Winchester, 2004), a considerable number of these conditions result from defects in lysosomal membrane proteins or non-enzymatic soluble lysosomal proteins (Saftig and Klumperman, 2009). Therefore, LSDs offer a window into the normal functions of both enzymatic and non-enzymatic lysosomal proteins.

Most lysosomal proteins are the products of housekeeping genes that are expressed throughout the body, but storage occurs only in those cells with an available substrate (e.g., GM2 ganglioside is present predominantly in the CNS and deficiency of hexosaminidase A, which acts on the GM2 ganglioside and can be measured in the blood, causes Tay Sachs disease, a CNS condition). In all such cases, the diagnosis must be established by specific enzyme assays followed by mutation study of the gene.

1.2 Physiological considerations

1.2.1 Endosomal-lysosomal system

The lysosomes is just only one compartment consist of series of unconnected intracellular organelles, that is collectively known as endosomal-lysosomal system or

vacuolar apparatus, a term first coined by Christian by De Duve and Wattiaux (1966). Over 30 years ago, Novikoff (1973) described various compartments of the system. A confusing plethora of terms have been applied to the various compartments of this system. However, now generally it is accepted that its principal components are early endosomes located at cell periphery, late endosomes that tends to be perinuclear and the lysosome. They all combine and form a chain that is responsible for trafficking and digestion of endocytosed molecules. Until recently, it was considered to be the only function. However, over the time it has become clear that endosomes alsorepresents a major sorting compartment of the endomembrane system in cells.

The final component of this system is lysosome. De Duve et al. (1955) were the first to coin the term 'lysosome'. It is characterized by having membrane, low internal pH and vesicles containing many hydrolytic enzymes. The membrane of lysosomes contains transport system that carry particles between lumen and cytosol, and an electrogenic proton pump called the vacuolar proton pump or V-type H+-ATPase (Arai et al., 1993). It also contains several membrane proteins that are of uncertain function (Eskelinen et al., 2003). It is in the lysosome where substrate breakdown occurs. However it is considered as 'dead end' compartment. For example lysosomes are capable of functioning and secreting it contents after fusion with the plasma membrane (Luzio et al., 2000). Phagosomes on the contrary are formed by the phagocytosis of bacteria and cellular debris and it eventually transforms into phagolysomes. Lysosome has also other function of calcium regulated exocytosis of lysosomes needed for membrane repair (Reddy et al., 2001).

1.2.2 Lysosomal enzymes: Synthesis and Trafficking

The different steps that are involved in the synthesis of lysosomal enzyme are shown in Figure 1.1.The lysosomal enzymes are glycoproteins that are synthesized in rough endoplasmic reticulum (ER). At the early stage of process they are inactive. They translocate through the ER membrane to the lumen of ER with the help of N-terminal signal sequences. Once in the lumen of ER, they are N-glycosylated and their signal sequence is cleaved. They then move to the Golgi compartment, and at this stage they acquire mannose 6-phosphate (M6-P) ligand. In this process two enzymes sequential action is needed, a phosphotransferase (Reitman & Kornfeld, 1981; Waheed et al., 1981) and a diesterase (Varki & Kornfeld, 1981; Waheed et al., 1981). It is important

to understand mechanism of these two enzymes (Figure 1.2), as it is the acquisition of M6-P marker that separates glycoproteins which has to move to lysosomes from secretory glycoproteins. Failure of the acquisition of this marker leads to mistargeting of lysosomal enzymes and it will not be able to enter in lysosomes and substrate breakdown will not occur. Example of this mechanism is called as receptor deficiency resulting in the secretion of enzymes into an extracellular matrix giving rise variable complex phenotypes. This condition is known as mucolipidoses that is I-cell (mucolipidosis type II)and mucolipidoses-III. In these patients, there is lack of phosphotransferase enzyme that is responsible for the first step of mechanism as shown in Figure 2. Consequently all enzymes that requires M6-P marker for entering into the lysosomes fails.These observations led to the discovery of M6-P ligand and its receptor (Hickman & Neufeld, 1972). However, all lysosomal enzymes do not require M6-P marker and ligand. Glucocerebrosidase lysosomal enzyme that is associated with lysosomal membrane does not require MP-6 residue but in undergoes N-glycosylation process and is targeted to the lysosomes.

The receptor-protein complex then moves to late endosome, where dissociation occurs due to low pH (Gonzalez-Noriega et al., 1980). The hydrolase translocates into the lysosomes and then the receptor is either recycled to Golgi to pick up another ligand or to the plasma membrane Figure 1.3.The final step in maturation of the lysosomal enzyme includes proteolysis, folding and aggregation.

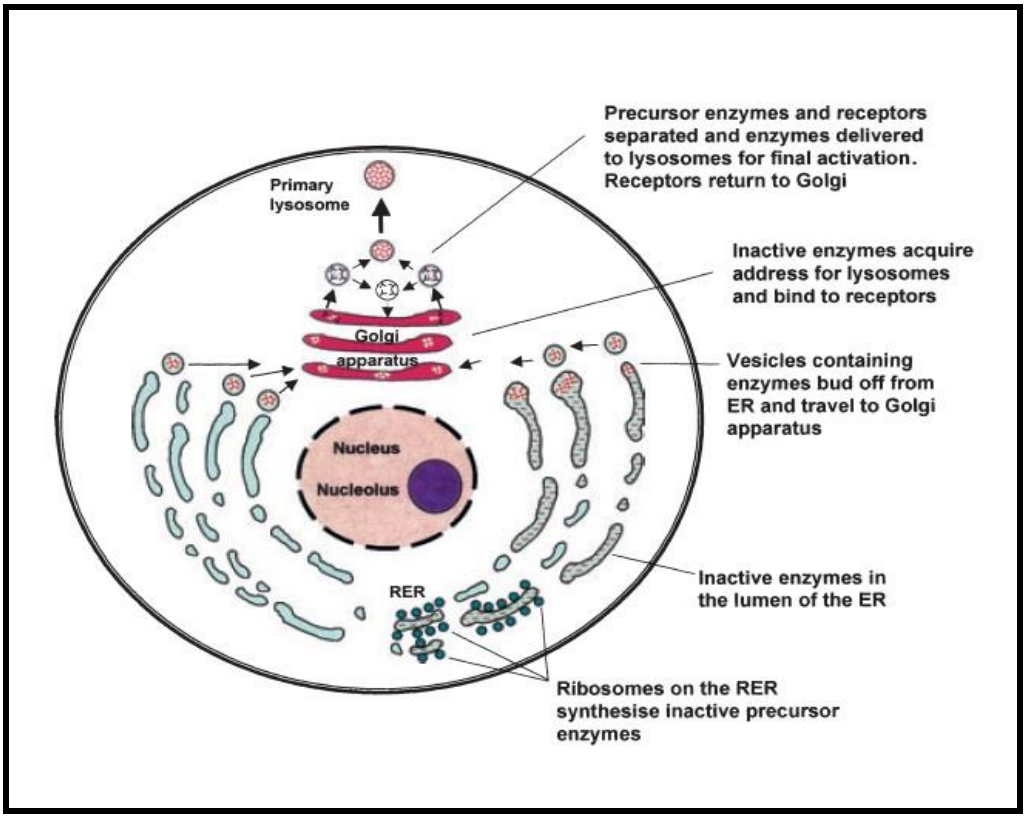

Figure 1.1: Making the lysosomal enzymes (Vellodi, 2004)

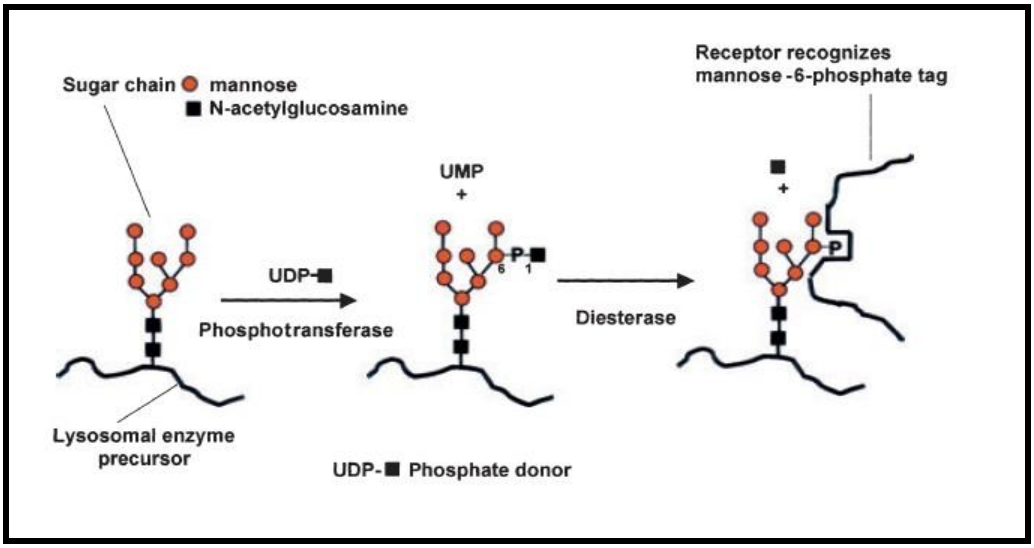

Figure 1.2: Formation of lysosomal recognition tag or marker, mannose-6-phosphate (Vellodi, 2004)

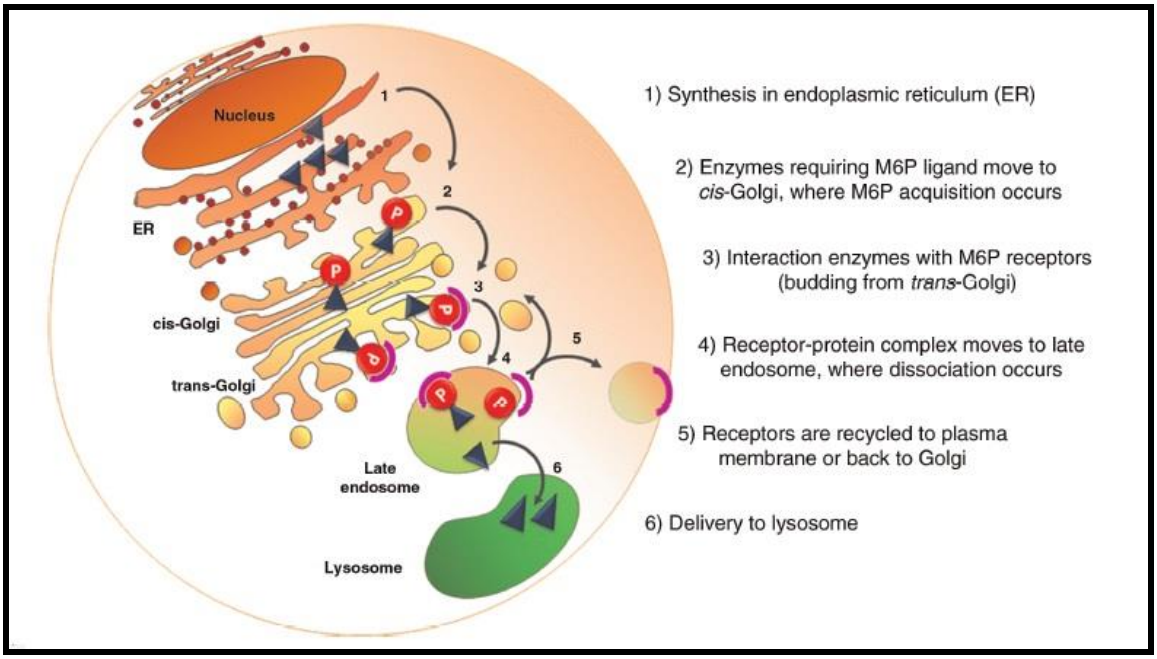

Figure 1.3: Simplified scheme of M6P-dependent enzymes sorting to the lysosome. The enzyme UDP-N-acetylglucosamine-1-phosphotransferase, responsible for the initial step in the synthesis of the M6P recognition markers, plays a key role in lysosomal enzyme trafficking. Loss of this activity results in mucolipidoses II/III. Note that not all lysosomal enzymes depend on the M6P pathway (Filocamo and Morrone, 2011)

1.2.3 Endocytotic pathway: Transport of macromolecules to the Lysosome

The steps of endocytic pathway are shown in Figure1.4.The material that has to be broken into lysosomes may be intracellular or extracellular. Extracellular materials enter the cell either by endocytosis or phagocytosis dependingon the nature of the molecule. Receptor mediated endocytosis is the process by which most biologically important extracellular substances are internalized and this occurs by binding to specific cell surface receptors (Goldstein et al., 1985).

Ligands are first transported to the early endosmes and from there it moves to late endosomes probably by multivesicular bodies. Finally they are delivered to lysosomes. Phagocytosis is the process in which there is a route entry of microorganisms and cellular debris into the cell. Such particles are then incorporated into phagosomes, which fuse with primary lysosomes to form secondary lysosomes. Finally, intracellular materials undergo autophagy.

The small amount of hydrolysis takes place in endosomes, the bulk of its take place in the lysosomes.It is only in the acid milieu of the lysosome that hydrolases are active.

The vacuolar proton pump maintains the low pH of the lysosomes.

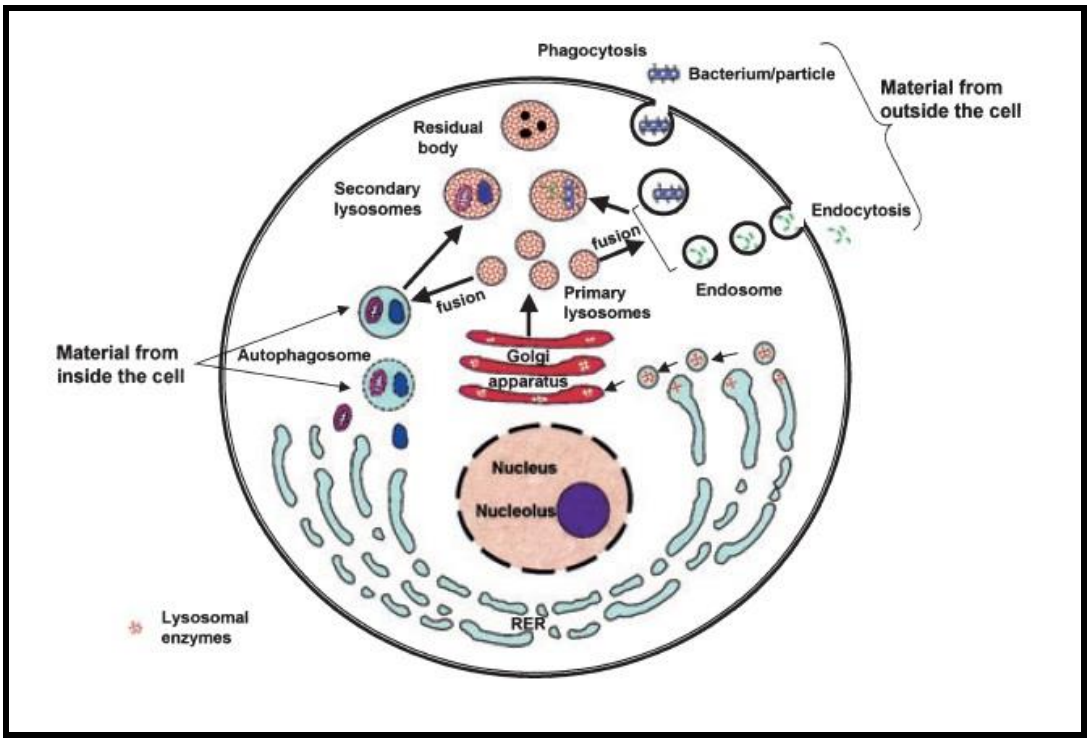

Figure1.4: The endocytic pathway (Vellodi, 2004)

1.2.4 Endosomal–autophagic–lysosomal function and dysfunction in storage diseases

Lysosomes play a central role in processing the clearance of cellular substrates from multiple routes within the endosomal– autophagic–lysosomal system Figure 1.5. It isacidic organelles that contain enzymes required for the degradation of macromolecules, and efflux permeases that facilitate the inside-out translocation of small molecules that are generated through macromolecule catabolism. It is smaller in size in comparison to endosomes and autophagosomes.They are highly enriched in particular transmembrane proteins and hydrolytic enzymes (including proteases, glycosidases, nucleases, phosphatases, and lipases), have a higher buoyant density, an electron- dense appearance by transmission electron microscopy, and a high proton and Ca2+ content (Luzio et al., 2007; Saftig and Klumperman, 2009; Morgan et al., 2011). It differs from endosomes in their degree of acidification and more abundant levels of lysosomal membrane proteins (LMPs) such as LAMP1 and LAMP2. Most of

the nascent lysosomal enzymes bind to mannose-6-phosphate receptors (M6PRs) in the trans-Golgi network (TGN), which then traffic the enzymes to early and late endosomes (Ghosh et al., 2003). Lysosomes in turn receive these enzymes back when endosomal–lysosomal fusion occurs. When lysosomes are dense, notably it does not contain M6PRs. Acidotropic reagents such as Lysotracker are useful for labeling lysosomes. However, the interior of late endosomes is mildly acidic and autophagosomes also allows Lysotracker to label these organelles to varying degrees (Bampton et al., 2005).

The biogenesis and functioning of endosomal and auto-phagosomal pathways is controlled by transcription factor EB (TFEB), which regulates the expression of 471 genes that constitute the CLEAR (coordinated lysosomal expression and regulation) gene network (Sardiello et al., 2009; Palmieri et al., 2011). Degradation of endosomal and autophagosomal material takes place upon exchange of content (via transient "kiss-and-run"contacts) or fusion with lysosomes, forming endolysosomes (Tjelle et al., 1996; Bright et al., 1997, 2005; Mullock et al., 1998) and autolysosomes (Jahreiss et al., 2008; Fader and Colombo, 2009; Orsi et al., 2010), respectively (Figure 5, A and B). Lysosomes are considered as storage compartments for acidic hydrolases that enter cycles of fusion and fission with late endosomes and autophagosomes.While the digestion of endocytosed and autophagic substrate takes place primarily in endolysosomes and autolysosomes (Tjelle et al., 1996; Luzio et al., 2007). Under physiological conditions, endolysosomes and autolysosomes are transient organelles.

Cells that are deficient in lysosomal hydrolytic enzymes, lysosomal membrane proteins, or non-enzymatic soluble lysosomal proteins accumulate excessive levels of undegraded macromolecules (enzyme deficiency) or monomeric catabolic products (efflux permease deficiency) and contain numerous endo/autolysosomes Figure 1.6. Presence of high levels of macromolecules/monomers in endo/autolysosomes, catabolic enzymes are inhibited and permeases that are not genetically deficient, which results in secondary substrate accumulation (Walkley and Vanier, 2009; Lamanna et al., 2011; Prinetti et al., 2011). For example, lysosomal proteolytic capacity is reduced in fibroblasts from various LSDs, such as mucopolysaccharidoses I and VI, and GM1-gangliosidosis, which themselvesare not caused by protease deficiency (Kopitz et al., 1993). The accumulation of primary and secondary substrates sets off a cascade of events that impacts not only the endosomal–

autophagic–lysosomal system, but also other organelles, including mitochondria, the ER, Golgi, peroxisomes and overall cell function (Figure 1.7).

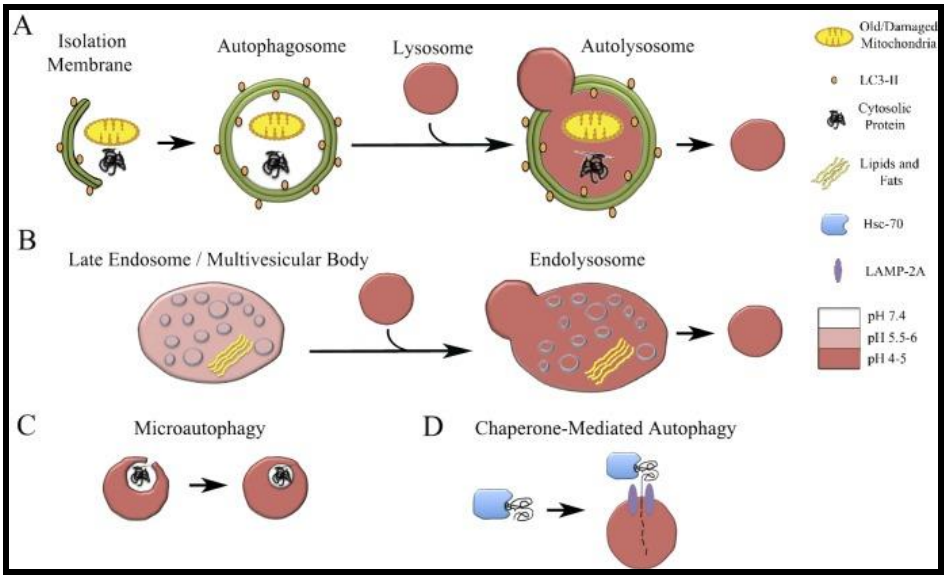

Figure 1.5:Lysosomes as catabolic centers of the cell. Lysosomes utilize four distinct pathways for the degradation of cellular material. (A) Macroautophagy begins with the formation of isolation membranes that sequester regions of the cytosol that include denatured proteins, lipids, carbohydrates, and old/damaged organelles into encapsulated vesicles known as autophagosomes. The dynamic kinetics of autophagosome production and clearance by lysosomes is known as autophagic flux. (B) Endosomal degradation by lysosomes predominantly targets late endosomes/multivesicular bodies. Fusion between late endosomes and lysosomes can occur by (i) full fusion/degradation or (ii) kiss-and-run content mixing, where transient endosomal docking occurs. (C) Microautophagy involves the pinocytosis of cytosolic regions surrounding lysosomes. (D) Chaperone-mediated autophagy (CMA) selectively targets proteins with a KFERQ motif for delivery to lysosomes using Hsc-70 as its chaperone and LAMP-2A as its receptor (Platt, 2012)

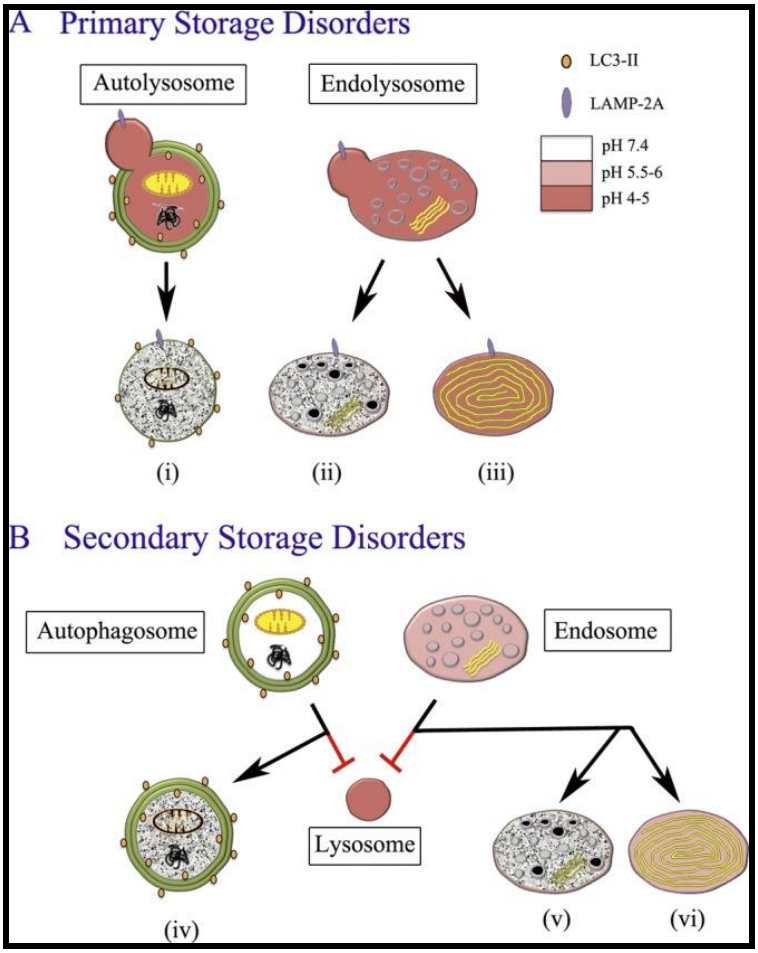

Figure 1.6: Subtypes of storage organelles accumulate in LSDs. In different LSDs, cells display a unique spectrum of dysfunctional organelles depending on the specific lysosomal enzyme or non-enzymatic protein affected. (A) In primary LSDs, deficiencies in degradative enzymes prevent the clearance of autophagic and endocytic substrates, resulting in the accumulation of (i) autolysosomes (LC3-II (+), LAMP-1 (+)), (ii) endolysosomes (CI-MPR (+), LAMP-1 (+)), and (iii), in the case of certain lipase deficiencies, lipid-rich multilamellar bodies (CI-MPR (+), LAMP-1 (+)). (B) In a secondary storage disease such as Niemann-Pick type C1, lysosomal enzyme function remains intact, but impaired heterotypic fusion of autophagic and endocytic organelles with lysosomes results in the accumulation of (iv) autophagosomes (LC3-II (+), LAMP-1 (-)), (v) late endosomes (CI-MPR (+), active cathepsin D (-)), and (vi) endosome- derived multilamellar bodies (lipid-rich, CI-MPR (+), active cathepsin D (-)) (Platt, 2012)

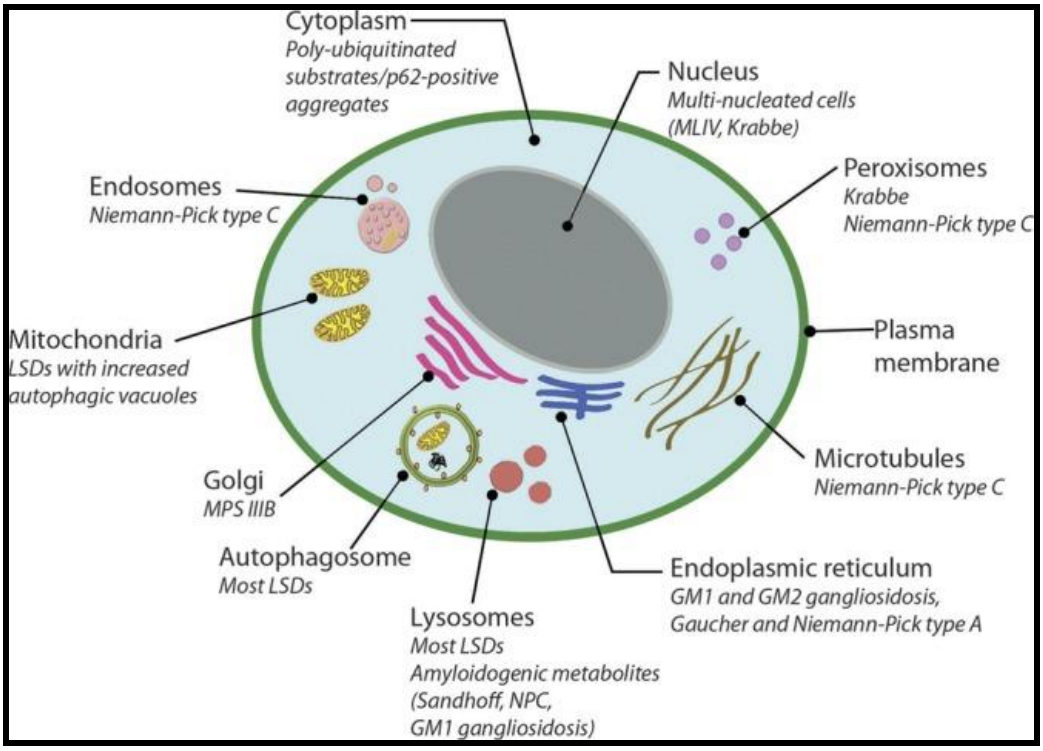

Figure 1.7: Summary of organelles affected in LSDs (Platt, 2012)

1.2.5 Secretion–recapture pathway

A significant proportion of newly synthesized enzyme is not bound to the M6-P receptor in the Golgi but instead is secreted and then endocytosed into neighbouring cells via M6-P receptors on the plasma membrane (Vladutiu & Rattazzi, 1979). This secretion–recapture pathway iscrucial in the understanding of the mucolipidoses types II and III. These disorders are characterized by grossly elevated extra lysosomal (plasma and cytosol) levels of a large number of lysosomal enzymes that require the M6-P recognition marker for receptor-mediated uptake. This was subsequently proven that they arecharacterized by failure of enzymes to acquire this recognition marker.

The concept of M6-P-based secretion and recapture is of considerable importance when considering therapy. However, there is now evidence that, for some lysosomal enzymes atleast, this process is independent of this receptor (Muschol et al., 2002).

1.3 Pathology of Lysosomal Storage Disorder (LSD)

1.3.1 Causes of LSD

There are many steps in the synthesis and processing of lysosomal hydrolase and many ways by which lysosomal enzyme dysfunction occurs. Since the discovery of first lysosomal enzyme deficiency for Pompe's disease (Hers, 1963) made, over 40 LSDshave been described. There may beinherent defects of synthesis or folding, activator protein defects as in the saposin deficiencies, targeting defects as in mucolipidoses II and III, transporter defects like Niemann–Pick C disease and membrane protein defects as in cystinosis and, infantile sialic acid storage disease.

1.3.2 Relationship with residual enzyme activity

The residual activity of enzyme is very closely related to the severity of phenotypes. In 1983, Conzelmann and Sandhoff proposed that there was a 'critical threshold' of enzyme activity. Above this level enzyme activity can deal with substrate influx. Below this, it cannot and lead to accumulation of substrate. It has been observed that even small change in residual activity have profound effect on the rate of substrate accumulation (Conzelmann & Sandhoff, 1983). In general, the lower the residual activity, the earlier the age of onset and are severe form of the disease, although there is considerable overlap, for example, in Gaucher's disease. Gaucher disease is the classical example with single enzyme deficiency leading to three different phenotypes. Therefore, for the diseases in which enzyme based therapies are available it is of critical importance to monitor the response of treatment by biomarkers and using several clinical end points.

1.3.3 Effects of substrate on lysosomal morphology

The buidup of undigested material secondary to the lysosomal enzyme dysfunction results in the formation of typical histochemical and ultrastructural changes.Light microscopy often shows engorged macrophages with a characteristic appearance, such as that of 'crumpled silk' in Gaucher's disease (Parkin & Brunning, 1982) or 'sea-bluehistiocytes' in Niemann–Pick disease (Vanier et al., 1988). Characterstics in ultrastructure changes also have been observed on the basis of appearance of residual bodies. These are the vacuoles containing undigested materials, and are the hallmark

of primary storage in these disorders. The first residual bodies were observed in Tay–Sachs disease (Terry & Weiss, 1963). Other disorders in which they have been described includeneuronal ceroid lipofuscinosis (Zeman & Donahue, 1963) in Batten disease, spingolipids in Gaucher's disease (Lee, 1968) and oligosaccharides in fucosidosis (Loeb et al., 1969; Freitag et al., 1971).

1.3.4 Effect of accumulated substrate on Phenotypes

There are several ways in which accumulation of substrate resulting in the dysfunction of the organ where it is stored (Figure 1.8).The most common is enlargement of the affected cell that results in the enlargement of respective organ. For many years it was considered that manifestation of hepatosplenomegaly, cardiomyopathy etc was solely due to undegraded substrate accumulation. Later on number of secondary biochemical and structural events have been reported which appears to be triggered by the primary storage event. All these factors, either individually or in combination, appear to play key roles in the pathophysiology of LSD.

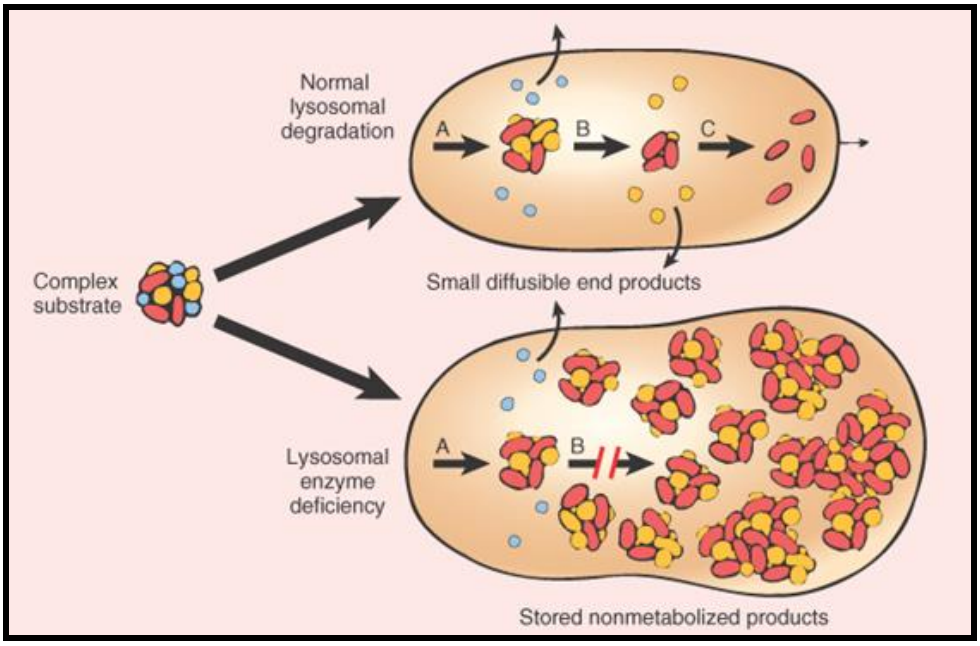

Figure 1.8: Effect of lysosomal enzyme deficiency on cell morphology

1.4 Clinical phenotypes of LSDs

The accumulation of undegraded cellular debris is progressive, ultimately causing deterioration of cellular and tissue function. This leads to the activation of a variety of pathogenetic cascades that result in complex clinical pictures that are characterised by multisystemic involvement (Ballabio and Gieselmann, 2009; Wraith, 2002; Futerman and Van Meer, 2004; Vellodi, 2005; et al., 2010).

Phenotypic expression is extremely variable, as it depends on the specific macromolecule accumulated, the site of production and degradation of the specific metabolites, the residual enzymatic expression and the general genetic background of the patient. Many LSDs have phenotypes that have been classified as infantile, juvenile and adult (Wraith, 2002). Many disorders affect the central nervous system (CNS) and most patients have a decreased lifespan and significant morbidity. The LSDs are often categorized according to the type of substrate stored (i.e. mucopolysaccharidoses, oligosaccharidoses, sphingolipidoses, gangliosidoses, etc.) (Wilcox, 2004).

The age of clinical onset and spectrum of symptoms exhibited amongst different LSDs vary, depending on the degree of protein function affected by specific mutations, the biochemistry of the stored material, and the cell types where storage occurs. Apart from lysosomal diseases involving substrate storage in bone and cartilage (e.g., the mucopolysaccharidoses) most babies born with these conditions appear normal at birth. The classical clinical presentation of LSD is a neurodegenerative disease of infancy/childhood (Wraith, 2002), but adult- onset variants also occur (Spada et al., 2006; Nixon et al., 2008; Shapiro et al., 2008). Relatively some lysosomal diseases lack pathology in the central nervous system (CNS) (Wraith, 2004). In the majority of LSDs, CNS involvement is common and neurodegeneration can occur in multiple brain regions (e.g., thalamus, cortex, hippocampus, and cerebellum). Neuropathology in LSDs involves unique temporal and spatial changes that often affect early region-specific neurodegeneration and inflammation, before global brain regions are affected. The main reasons for these are: threefold: (1) specific storage metabolites exert differential effects on neuronal subtypes, (2) varying proportions of macromolecules are synthesized in different neuronal populations, and (3) there is differential neuronal vulnerability to storage (e.g., Purkinje neurons degenerate in many of these diseases

leading to cerebellar ataxia). In LSDs, activation of the innate immune system is also prevalent in the brain, which directly contributes to CNS pathology (Vitner et al., 2010). Another common feature of LSDs is astrogliosis (activation of astrocytes) that damages neurons through an inflammatory process known as glial scarring (Jesionek-Kupnicka et al., 2003; Vitner et al., 2010).

A notable non-neuronopathic LSD is Type 1 Gaucher disease (β-glucocerebrosidase deficiency), which is relatively most common LSD, particularly within the Ashkenazi Jewish community. The major cell type affected by glucosylceramide storage in this disease is the macrophage ("Gaucher cells"), whose dysfunction affects the production and turnover of cells belonging to the hematopoietic system. Infiltration of Gaucher cells occurs in various organs and affects the immune system, bone strength, spleen, and liver function.

A key question currently challenging in this field is how endosomal–lysosomal storage leads to pathogenesis and how expanding this knowledge will help to improve treatment for patients (Bellettato and Scarpa, 2010; Cox and Cachón-González, 2012). This review aims to delineate regulatory systems and organelles that become disrupted in these disorders, highlighting the complexity of cellular storage, its consequences on pathogenesis, and its implications for therapy.

1.5 Classification of LSD

LSDs can be grouped according to various classifications. In the past, they were classified on the basis of the nature of the accumulated substrate(s), more recently they have tended to be classified by the molecular defect. A classic example of LSDs grouped by storage is the group of mucopolysaccharidoses, that results from a deficiency of any one of 11 lysosomal enzymes that are involved in the sequential degradation of glycosaminoglycans (or mucopolysaccharides). In the group of sphingolipidoses, undegraded sphingolipids accumulate due to an enzyme deficiency or to an activator protein defect. Among the oligosaccharidoses (also known as glycoproteinoses), a single lysosomal hydrolasedeficiency causes storage of oligosaccharides. In some cases, a deficiency of a single enzyme can result in the accumulation of different substrates. For example, GM1 gangliosidosis and Morquio-

B disease are both caused by an acid b-galactosidase activity defect, yet results in GM1 ganglioside and keratan sulphate accumulation, respectively. The emerging classification of diseases arebased on the recent understanding of the molecular basis of LSDs.

This subset includes groups of disorders due to: (i) non-enzymatic lysosomal protein defects; (ii) transmembrane protein defects (transporters and structural proteins); (iii) lysosomal enzyme protection defects; (iv) post-translational processing defects of lysosomal enzymes; (v) trafficking defects in lysosomal enzymes; and (vi) polypeptide degradation defects. Finally, another group includes the neuronal ceroid lipofuscinoses (NCLs), which are considered to be lysosomal disorders, even though distinct characteristics exist. While, in the classic LSDs, the deficiency or dysfunction of an enzyme or transporter leads to lysosomal accumulation of specific undegraded substrates or metabolites, accumulating material in NCLs is not a disease-specific substrate but the subunit of mitochondrial ATP synthase or sphingolipid activator proteins A and D (Jalanko and Braulke, 2009).

Multiple sulphatase deficiency (MSD) is also worth mentioning. It has been shown that MSD results froma post-translational processing defect due to the failure of Ca-formylglycine-generating enzyme to convert a specific cysteine residue, at the catalytic centre of all sulphatases, to a Ca-formylglycine residue (Cosma et al., 2003, Dierks et al., 2003). Another rare LSD, galactosialidosis, is associated with the defective activity of two enzymes, b-galactosidase and sialidase. In these diseases classified as ‘lysosomal enzyme protection defects’ a multi-enzyme complex between the two lysosomal enzymes and the protective protein, cathepsin A (PPCA), forms improperly (D’Azzo et al., 2001). The breakdown of certain glycosphingolipids by their respective hydrolases requires the presence of activator proteins, known as sphingolipid activator proteins or saposins, encoded by two different genes. The defective function of the GM2 activator protein results in the AB variant of GM2 gangliosidosis (Conzelmann and Sandhoff, 1983). The prosaposin is processed to four homologous saposins (Sap A, Sap B, Sap C and Sap D) (O’Brien et al., 1988). Deficiency of Sap A, Sap B and Sap C results in variant forms of (i) Krabbe disease, involving abnormal storage of galactosylceramide (Spiegel et al., 2005); (ii) metachromatic leucodystrophy (MLD), associated with sulphatide storage (Wenger et al., 1989); and (iii) Gaucher’s disease, involving glucosylceramide storage

(Christomanou et al., 1989) respectively. Rarely, a total deficiency of prosaposin has been reported, resulting in a very severe phenotype (Bradova, 1993). Mucolipidoses result from defects in the signaling enzymeUDP-N-acetylglucosamine-1-phosphotransferase, which plays a key role in lysosomal enzyme trafficking (Kornfeld and Sly, 2001). This enzyme is responsible for the initial step in the synthesis of the M6P recognition markers essential for receptor-mediated transport of newly synthesised lysosomal enzymes to the endosomal/ prelysosomal compartment (Figure 1.1). Failure to attach this recognition signal leads to the mistargeting of all lysosomal enzymes that require the M6P marker to enter the lysosome (Filocamo and Morrone, 2011).

1.6 Gaucher disease (GD)

Gaucher disease (GD) is the most common lysosomal glycolipid storage disorder characterized by the accumulation of glucosylceramide (glucocerebrosidase) as has been reported in Indian subjects (Sheth et al., 2013). The eponym GD encompasses the heterogenous sets of signs and symptoms in patients with defective intracellular hydrolysis of glucosylceramide and related glucosphingolipidsthat cleaves the glycolipid glucocerebroside into glucose and ceramide. It is an autosomal recessive inherited disorders result from mutations in *GBA* gene that encodes the lysosomal hydrolase, acid β-glucosidase or glucocerebrosidase (E.C. 3.2.1.45).

1.6.1 History

In 1882, a French medical student named Phillipe Charles Ernest Gaucher (1854 – 1918) in his doctoral thesis provided the first description of the disease, which was named "**Gaucher's disease**" by Brill in 1905 (Beutler and Grabowski, 1995). He described a 32- year- old female with an enlarged spleen that he thought was an epithelioma (Gaucher, 1882). He noted the presence of large, unusual cells in the patient's spleen. From 1895 to 1910 the morbid anatomy in children and adults was delineated and the systematic nature of disease was recognized. The reports of other patients with similar presentations appeared shortly thereafter, and the eponym 'Gaucher's disease' was applied to these individuals. The "Gaucher cell" term was used most commonly to specify the characterstic-engorged cells in the organ of these patients. In the beginning of this century GD was suggested as a familial disorder

(Brill, 1901) and 3 years later involvement of the lymph nodes, the liver and bone marrow was reported (Brill, 1904). One year later (Brill et al., 1905) made the first pre-mortem diagnosis of the patient with this disorder. It was quickly realized that the age at which signs and symptoms associated with Gaucher's disease become manifest varied greatly. In an infant; neurological impairement was first reported 6 years later (Oberling and Woringer, 1927). This phenotype eventually becomes known as the infantile or acute neuronopathic form and currently nomenclature for this type of patient is Type -2 GD. In the latter patients, during the pre-teen and early-teen years other neurological signs appear and phenotypes are called juvenile or chronic neuronopathic form and these types of patients are called Type -3 GD. However, the most GD patients that are most commonly found do not have signs of central nervous involvement. These patients were previously called adult GD but now they are designated as Type -1 GD (Zimran, 1997).

In 1924, Lieb identified accumulating material as a sphingoglycolipid that he erroneously believed to be galactocerebroside (Lieb, 1924). Glucocerebroside as the accumulating material was demonstrated by Aghion in 1934. Glucocerebroside is comprised of three components. The first is the amino alcohol called sphingosine. A long chain fatty acid is linked to the nitrogen atom on carbon two of sphingosine forming a complex called ceramide. A single molecule of galactose is linked by a glycosidic bond to the oxygen on carbon one of the sphingosine portion of ceramide.

1.6.2 Clinical Classification

Clinically, three major types of GD have been delineated based on the absence (type 1) or presence and severity (types 2 and 3) of primary central nervous system involvement. For avoiding non-specific description like "adults", "infantile", and "juvenile", the following classification has been adopted: type 1- nonneuronopathic; type 2- acute neuronopathic; type 3- subacute neuronopathic. However, within each type, even within the same ethnic and/or demographic groups, the phenotypes and genotypes can be markedly heterogenous.

Among all these, the most common form of the disease is type1, which is characterized by the lack of neuronopathic involvement. This type of GD is sometimes referred as the "adult" form. Although adult onset is common, onset in early childhood is the rule in type-1 patients.

Type-2 disease, the infantile form of the disorder, is characterized by infantile onset and severe neurological involvement.While type 3, the juvenile form of the disease, is characterized by neuronopathic disturbances that usually begin later in the first decade of life and progress more gradually than in the acute neuronopathic form of the disease. The distinction between severe type 1 and type 3 disease is often not obvious in early childhood, although there are certain genotypes such as N370S (1226A>G) is most common alleles in GD type-I patients and L444P (1448T>C) in type-2 and type-3 patients that are commonly associated with juvenile disease. Genotyping may provide classification of patients (Beutler and Grabowski, 1995).

1.6.2.1 Type 1 Gaucher disease (GD) (nonneuronopathic)

The clinical manifestations of type-1 GD result from engorged macrophages causing enlargement and dysfunction of the liver and spleen known as hepatosplenomegaly, displacement of normal bone marrow by engorged storage cells, and damage to bone lead to infarctions and to fractures. Sometimes there is involvement of other organs such as lungs, CNS (type II and III), heart, eyes, skin, bone marrow that contributes to the overall clinical picture (Nagral, 2014).

Type 1 GD has a broad spectrum of severity. At one extreme, patients may be diagnosed as late as the eighth and even ninth decade of life. Some asymptomatic patients with disease are recognized only during the course of family studies or during population surveys. Such patients are mildly affected and almost invariably found homozygous N370S (1226 A>G) missense mutation. The median age of first patients with clinical symptoms and this genotype was found to be 33 years. Although there are many such patients with this genotype had never come to medical attention at all.

At the other opposite extreme of type 1 GD are children with massive hepatosplenomegaly associated with severe abnormalities of liver function, pancytopenias and extensive skeletal abnormalities. Such children with these complications of GD may die at the first or second decade of life. Almost their genotype invariably includes atleast one deficient allele such as L444P (1448T>C) or 84GG mutation producing a frameshift in the leader sequence.

Some patients are with severe visceral (liver and spleen) enlargement and have minimal skeletal involvement, while some other patients with severe bone disease and

have minimal visceral enlargement. In other patients, visceral and skeletal involvements are approximately in equal severity. The type of mutation seems to have less effect on sites of disease involvement rather than on overall disease severity.

Children with type 1 GD shows growth retardation but their mental development is quite normal.

1.6.2.1.1 Hematologic Manifestations

In GD, bleeding is common presenting symptom of patients. The most common cause of this is probably thrombocytopenia. Deficiencies of factor XI are prevalent, but may be coincidential because a deficiency of this clotting factor is common in Ashkenazic Jews, the population that is most frequently affected by GD. In patients, who have been known to have GD for some years have bleeding as a much less common problem probably because of the ameliorating effect of splenectomy.

Thrombocytopenia is the most common peripheral blood abnormality. Early in the course of the disease it is usually due to splenic sequestration of platelets and invariably responds to splenectomy. Later in the course of the disease, in patients who have already undergone splenectomy, replacement of the marrow by Gaucher cells may be more important. Anemia is usually mild, but occasionally is quite severe, with hemoglobin level as low as 5 g/dl. Leukopenia also occurs in some patients. These changes are probably due to combination of increased splenic sequestration, when the spleen is present and decreased production because of replacement of the marrow by Gaucher cells (Beutler and Grabowski, 1995).

1.6.2.1.2 Spleen

Splenic disease is present in at least 95% of GD patients (Kolodny, 1982). Splenic enlargement is present in all but the very mildest cases of GD. Even in aymptomatic patients, it is commonly the presenting sign. Although nearly normal sized spleens can be present in asymptomatic patients, splenomegaly is present in all symptomatic patients and can be examined by physical examination or scanning procedures. In severely affected patients the spleen may be huge, filling the abdomen. The bulk of the spleen may interfere with normal food intake and it may cause dyspareunia in women. As in other states in which splenomegaly occur, splenic infarctions occur. In rare circumstances, large parts (25 to 50 percent) of a massively enlarged spleen can

infarct. Such patients present with an acute abdomen and fever, metabolic acidosis, and hyperuricemia. Selective splenic nuclide scans can be useful in delineating the presence and extent of new or old infarcts (Beutler and Grabowski, 1995).

The spleen may be just palpable below the coastal margin or, in case of early onset disease, which greatly distend the abdomen and extend into the pelvis. A medial notch may be readily palpable in this situation (Zimran, 1997).

Acute pain in the left hypochondrium or central abdomen heralds the onset of splenic infarction, a frequent accompaniment of the massive splenomegaly associated with GD. Local pain and tenderness is associated with fever and occasionally abdominal guarding. The spleen is often tender with evidence of perisplentis with or without peritonitis indicated by rebound tenderness and the presence of friction rub heard during respiration. Large splenic infarcts are frequently accompanied by abnormalities of serum liver-related enzymes and unconjugated hyperbilirubinemia, there may also be reduction in the already low platelet count and laboratory indications of consumptive coagulopathy. Other indications include cytopenia. The operation is not hazardous and partial splenectomy has been considered especially in infants (Zimran, 1997).

1.6.2.1.3 Liver

Liver enlargement is the rule in patients with GD. When the disorder is severe, the liver may fill the entire abdomen. On physical examination the liver is usually firm and smooth, and the right and left lobes are uniformly enlarged. Massively enlarged livers are usually hard and can have irregular surfaces. Frank hepatic failure and/or cirrhosis with portal hypertension and ascites are uncommon but do occur in small percentage of patients and bleeding from varices may cause death. The bulk of liver may cause distress to the patient, and the episodes of pain may occur. In patients with severe disease liver function abnormalities may be quite serious (Beutler and Grabowski, 1995).

The Kupffer cell or liver histiocyte is a specialized macrophage and microscopic examination indicates that it is an invariable site of abnormal glycolipid storage in Type-I GD. Clinical examination shows hepatomegaly in 70-80% of patients with Type-I Gaucher disease. However, clinically significant complications of hepatic

disease are rare and occur in less than 5% of affected patients. Hepatic infarction is well recognized in GD with clinical features resembling those of splenic infarction: fever, pain in the right flank and sub-coastal region associated with signs of peritoneal inflammation accompanied by a friction rub are characteristic. Hepatic infarction appears to have been particularly frequent and accompany the rapid increase in peripheral platelet count in the immediate period after splenectomy. Recurrent infarction of the liver appears to cause extensive scarring throughout the organ giving rise to a distorted appearance observed at surgery or at necropsy (Zimran, 1997).

1.6.2.1.4 Bone marrow disease

Diffuse involvement of the mononuclear phagocyte system including fixed tissue macrophages in the liver, spleen and bone marrow, has rendered it difficult to determine the individual effects of Gaucher's cell infiltration on the bone marrow in those patients in whom the spleen remains intact. In Type-I GD, the presence of sheets of Gaucher's cells within the marrow space associated with pericellular fibrosis and an increased deposition of reticulin leads to difficulties in aspiration biopsy at diagnosis. In the presence of a greatly enlarged spleen the contribution of marrow disease to the wide-ranging haematological abnormalities observed in GD is unclear. Characteristically, GD is associated with mild-to-moderate macrocytic anaemia, reflecting intrinsic marrow disease and associated with thrombocytopenia and leukopenia, splenectomy leads to agreat increase in platelet count and the appearance of target cells, Howell Jolly bodies, lymphocytosis and monocytosis (Zimran, 1997).

1.6.2.1.5 Lungs

Although it is relatively uncommon, pulmonary failure is one of the most serious consequences of GD. It may occur as the result of frank infiltration of lung by Gaucher cells, particularly in children, but severe pulmonary failure also occurs in patients without demonstrable Gaucher cells in the lungs. In such patients right-to-left intrapulmonary shunting, probably secondary to liver disease, seems to be the cause. Clubbing is generally a feature of this complication (Beutler and Grabowski, 1995).

1.6.2.1.6 Cardiopulmonary system

In patients with GD, clinically significant cardiopulmonary disease is a very rare and poor prognostic sign. The dyspnea on exertion and tachypnea frequently observed in

affected children with massive hepatosplenomegaly are usually due to limited diaphragmatic excursions. In GD, heart involvement was primarily reported in two adult females with Gaucher cell infiltration of the myocardium. In our study, one 4-year child X-ray of chest posterior anterior showed cardiomegaly with pulmonary congestion (Sheth et al, 2011).Percarditis of undetermined etiology wasalso seen. These were probably due to intercurrent illness, since even the most severe type-2 patients have not had cardiac or pericardial involvement. In adults, pulmonary hypertension has been related to Gaucher cells plugging the alveolar capillaries or infiltration of lung parenchyma. In adult patients with extensive hepatic involvement, cyanosis and clubbing can result from shunting within the lung. It has been suggested that this may be caused by an unidentified humoral factors that disappears following hepatic transplantation. Such right-to-left shunting has resulted in a pyogenic cerebral absence in one adult. Pulmonary infiltration of alveolar capillaries and frank consolidation of alveoli by Gaucher cells that could have been a contributory cause of death in these neurologically impaired children. Three patterns of pulmonary pathology have been observed in a large autopsy series: (1) interstitial Gaucher-cell infiltration with fibrosis, (2) alveolar consolidation and filling of alveolar spaces by Gaucher cells, and (3) capillary plugging by Gaucher cells and resultant secondary pulmonary hypertension. It also occurs in the absence of capillary plugging by Gaucher cells due to extensive thickening of medial and intimal layers of pulmonary arterioles.

The usual cardio-respiratory effects of GD result from compression of the lung caused by massive visceromegaly or thoracic restriction by kyphoscoliosis related to vertebral collapse. The radiological changes have included bilateral fine reticular opacification or a reticulo-nodular pattern; terminal alveolar disease is characterized by diffuse hazy opacification (Beutler and Grabowski, 1995).

1.6.2.1.7 Nervous system

GD as type-I was defined by the absence of primary central nervous system involvement. However, occasionally central nervous symptoms can be observed as a secondary manifestation of true type-I disease. Thus, massive sysyemic fat emboli involving the brain and lung, compression of the spinal cord secondary to vertebral collapse have also been observed (Beutler and Grabowski, 1995). Coagulopathies

have also been reported to cause central nervous system damage (Beutler and Grabowski, 1995). Central nervous system abnormalities without other known cause have been observed in patients with adult onset disease, but the relationship between symptoms and putative brain involvement in these patients was unclear.

The most striking feature is the presence of "Gaucher cells" in the perivascular Virchow-Robin spaces in the cortex and deep white matter. Not infrequently these periadventitial cells are also found in the gray matter of the thalamus and subependymal tissue of the pons and medulla. Although the exact origin of these cells appear to elicit gliosis and microglial proliferation in the brain parenchyma that approximates the perivascular gaucher cells, and local loss of neurons was demonstrated using antineurofilament antibody staining (Beutlerand Grabowski, 1995)

1.6.2.1.8 Bones

The skeletal manifestations of GD can be totally debilitating. On radiographic, scintigraphic, computed tomographic, or magnetic imaging scans, nearly all affected patients are shown to have bony lesions. The "Erlenmeyer flask deformity" of the distal femur is a common radiographic finding but is not universally present. Generalized bone loss has also been reported.

Many patients with radiographically significant GD of bone have few or no bony symptoms. However, in 20 to 40% of patients episodic excruciatingly painful "bone crises" occur. These crises are much more frequent in children and adolescents, but can occur for the first time in the third to seventh decades. The femoral head and the femoral shaft are by far the most frequently involved sites in a crisis, but these episodes occur with some frequency in the humeral heads, vertebral bodies, and ischium of the pelvis. The crisis occurs spontaneously or follows a febrile syndrome and begins with a dull, deep, aching pain in the involved bone. Over about 2 to 4 days the pain may become excruciating and difficult to control with analgesics. On X-ray, lesions may be absent, even though there is severe pain. However, areas of ischemia may usually be detected on Tc bone scans. Within a few days the intense pain begins to subside to a dull ache that can persist for several weeks. Recurrence is often at a different location.

In children, the acute hip lesions can be misinterpreted as Legg-Calve-Perthes disease, but this is rarely the presenting symptoms of GD. Involvement of the vertebral bodies is less common, but can lead to extensive collapse, gibbus formation, and spinal cord or nerve compression. Delayed collapse of vertebrae sometimes occurs after trauma. In experience reported, vertebral involvement with collapse occurs almost exclusively during the pubertal growth spurt, with rapid progression until the end of puberty. The aggressive phase of bony involvement is followed in adults by a more slowly progressive process or a cessation of active bony destruction as assessed by current radiographic and MRI techniques. Although pathological fractures of the femoral neck may occur in the third, fourth and later decades, these usually are in areas of bony lesions. Development or redevelopment of apparent Gaucher disease with vertebral, femoral, or other bony disease later in life is a clear indication to exclude malignancy, particular multiple myeloma, or infectious etiologies. Direct erosion through the cortical bone with extravasation of Gaucher cells and formation of sinus tracts has occurred spontaneously or following surgical intervention (Beutler and Grabowski, 1995).

The first abnormality is the loss of calcification of trabecular bone or osteopetrosis. The involvement of bone appears to progress through several stages, leading to severe destruction of the bone, joints, and medullary cavity. In GD, almost all bones have been involved, including in approximately decreasing frequency, the femoral necks and heads, femoral shaft, humeri, vertebral bodies, tibias, ribs, pelvis, bones of the feet, calvarium and mandible. The immediate cause of “bone crisis” is vascular compromise, infarction, and increased intramedullary pressure due to resultant edema. However, the events initiating the bone disease are very poorly understood, and few clues as to the pathophysiology of the bony disease are suggested by the gross or microscopic pathology. Microscopically, the earliest lesion appears to be increased reticulum fibers surrounding individual Gaucher cells. The finding of hyperemia leading to osteopenia and dilation of the Haversian canals suggests the presence of an inflammatory or toxic agent that initiates the pathological process. The lesions in bone include loss of trabeculae, fibrosis, necrosis, calcification of infarcted areas, and various stage of healing fractures. Complete obliteration of marrow by fibrosis and osteosclerosis can occur and progresses from proximal to distal with gradual replacement of bone marrow (Beutler and Grabowski, 1995).

Adult patient with GD develops osseous complications. The cause of bone disease is due to local infiltration by abnormal macrophages within the bone marrow. The simple mechanical effects of the Gaucher's tissue lead to an increase in intra-osseous pressure that combined with the local release of hydrolases and possibly cytokines contribute to the bone abnormalities. Generalized disorders of bone including osteopenia, or frank osteoporosis may result from systemic release of cytokines, particularly tumour necrosis factor and interleukin 6, which are known to be increased in some patients with GD and which are important promoters of osteoclastic resorpton. However, the lytic lesions are closely related to foci of GD in the intramedullary space. Painful bone crises are now known to result from episodes of bone infarction leading ultimately to bone death (avascular necrosis) otherwise known as 'osteonecrosis'. These changes clearly do not result from abnormal osteoclast activity but appear to result from microcirculatory disease of the bone leading to nutrition starvation of living osteones and bone death. The best-known skeletal manifestations of GD (the so- called Erlenmeyer flask deformities well known in the radiological literature) are in fact the least important. This condition occurs in up to 80% of patients and appears to have no prognostic significance in its own right. It affects the distal femur and proximal tibia and represents a clear failure of modeling of the metaphyseal regions leading to loss of normal development of tubulation during growth. It occurs due to the remodeling of the normally rounded ends of the bones into a flared, flattened shape (Lutsky and Tejwani, 2007) (Figure 1.9). Similar changes may be observed where osteoclast modeling and re-modeling is deficient, as in osteopetrosis. Eventhough it is a classical features of GD, the Erlenmeyer flask deformities is of little consequence. It nonetheless serves as pointer to the effects of GD on cell type such as osteoclasts, which are ultimately derived from common GM progenitor that gives rise to the pathological macrophage.

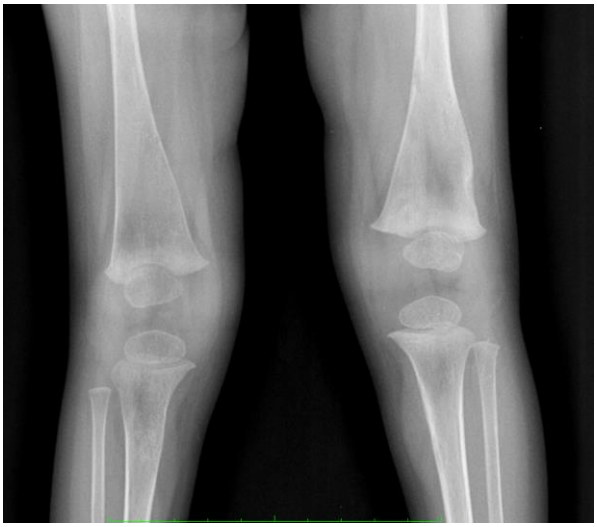

Figure 1.9:AP radiograph of GD patient. AP radiograph showing widening of the distal femur and proximal tibia, the "Erlenmeyer Flask" deformity, in apatient with GD

1.6.2.1.8.1 Osteopenia

A substantial decrease in bone density and cortical thinning is seen in almost all patients with GD (Mankin et al., 2001). Decreased bone density can be seen in the lumbar spine, femoral neck, trochanter, and distal radius. This is most apparent in patients who have previously undergone splenectomy (Pastores et al., 1996; Fiore et al., 2002). The femur is involved more often than the tibia with the epiphyses of the bones usually spared until the disease has advanced through the metaphyses and diaphysis (Tauber and Tauber, 1995).

Pathologic fractures may occur through compromised bone including femoral neck, thoracolumbar spine, and tibial plateau (Katz et al., 1987, 1993; Goldman and Jacobs, 1984; Seisheimer and Mankin, 1977; Kocher and Hall, 2000). Gaucher cell accumulation in bone marrow may cause small areas of bony erosions or large, soft, tumor-like masses, which may also be significant enough to cause pathologic fracture or deformity (Cotran et al., 1994; Barone et al., 2000).

1.6.2.1.8.2 Osteonecrosis

Osteonecrosis, also called avascular necrosis, is probably the most clinically significant and disabling skeletal manifestation in GD (Stowens et al., 1985; Pastores

and Einhorn, 1995). Osteonecrosis is bone death, believed to be secondary to ischaemia due to chronic infarction, and once the necrotic process starts, it cannot be reversed. It affects predominantly the femoral head, proximal humerus and vertebral bodies, and can result in fracture and joint collapse. (Wenstrup et al., 2002)

Patients with GD are at risk for the development of osteonecrosis, which may present in two forms. The first form, medullary osteonecrosis, is caused by occlusion of medullary blood vessels and may be asymptomatic. Subsequent death of the Gaucher cells creates an insoluble calcium soap, which leads to an area of increased medullary density on radiographs (Mankin et al., 2001). The second form involves corticocancellous structures, with the proximal and distal femur, proximal tibia, and proximal humerus most commonly affected (Mankin et al., 2001; Goldblatt et al., 1978; Rodrigue et al., 1999). Risk factors for osteonecrosis in GD include male gender, high platelet counts, and osteonecrosis in another location. Additionally, patients who have undergone prior splenectomy have been found to be ten times more likely to have osteonecrosis than patients who have not (Rodrigue et al., 1999).

1.6.2.1.9 Other Manifestations

In GD, skin problems are infrequent and nonspecific, such as diffuse brown or yellow-brown pigmentation and easy tanning. Severe neonatal ichthyosis (“collodion babies”) havebeen described in infants with acute neuronopathic GD but it is not at all clear that a cause-and-effect relationship existed between the glycolipid storage disease and the cutaneous disorder. At the cornoscleral limbus of the eye brownish masses of Gaucher cells have been reported. Gaucher cells have been found in a colonic polyp and the maxillary sinus. In GD, often fever occurs but not always in connection with bone crises (Beutler and Grabowski, 1995).

1.6.2.2 Type 2 Gaucher disease (GD) (acute neuronopathic)

It is a rare form with no ethnic predilection, is an acute neuronopathic form of the illness presenting during infancy. There is some heterogeneity in this group of patient but not as much as in GD type 3 (Zimran, 1997). It is a moderate degree of heterogeneity in the onset and course of even the acute neuronopathic form of GD. Early and late-onset varieties have been delineated, but their overall clinical courses are similar. The principal difference is in the time of onset. Indeed, GD has been

reported to manifest as hydrops fetalis.

Extensive visceral involvement with hepatosplenomegaly is the rule. Oculomotor abnormalities are often the first manifestations with the appearance of bilateral fixed strabismus or of oculomotor apraxia. This may result in the appearance of rapid head thrusts as an attempt to compensate when trying to follow a moving object. Hypertonia of neck muscles with extreme retroflexion of the neck, bulbar signs, limb rigidity, seizures, and sometimes choreoathetoid movements occur, although the latter are more common in type 3 disease (Beutler and Grabowski, 1995).

In addition to typical systemic signs of GD, a characteristic neurological syndrome develop that includes dysphagia, persistent head hyper extension, paralytic strabismus because of abducens nerve paresis, trismus generalized spasticity and psychomotor regression. Laryngospasm and apneic spells often occur in the later stages of the disease. Some patients develop myoclonus and generalized tonic – clonic seizures. Most infants with type 2 disease die within the first 2 years of life as a result of progressive brainstem dysfunction. Some patients have a later onset and slower disease course but with the same symptoms and outcome (Kyllerman et al., 1990; Beutler and Grabowski, 1995; Brady, 1996). A very rare and severe form exists that can be called congenital GD, and is characterized by skin abnormalities and hydrops fetalis at birth, with death rapidly ensuing.

1.6.2.3 Type 3 Gaucher disease (GD) (subacute-neuronopathic)

Type 3 GD is chronically progressive neuronopathic form of the illness that presents during infancy, childhood, adolescence or adulthood. The clinical features of this phenotype are more variable than those observed in Type 2 GD (Zimran, 1997). The severity of type 3 disease is intermediate, between that of types 1 and 2. Massive visceral involvement is usually present. Neurologic symptoms similar to those observed in type 2 disease are present but with a later onset and relatively lesser severity (Beutler and Grabowski, 1995).

The prototype of type 3 disease is the Norbottnian form, the clinical manifestations of which have been described in considerable detail. The median age of onset of symptoms is at 1 year. The first symptoms are usually the results of visceral involvement, with neurologic findings developing in half of the children during the

first decade of life. As, in type 2 disease, disorders of eye movement are the usual first symptoms, with the subsequent development of other neurologic manifestations such as ataxia (Beutler and Grabowski, 1995).

Atleast four subtypes are distinguishable on clinical grounds; the most thoroughly characterized group of patients presents with progressive myoclonic epilepsy in conjuction with a horizontal supranuclear gaze palsy. Dementia, ataxia and spasticity develop as the illness progresses over several years. Systemic signs of GD tend to be mild with death occurring as a result of progressive neurological deterioration (Winkelman et al., 1983). Initially somatosensory evoked potentials in these patients show giant cortical potentials that reflect decreased inhibitory input in the cerebral cortex. Some patients have an early onset of symptoms followed by a rapid course and death by the age of 2-3 years. Other patients have a much slower course and live into their 40's (Zimran, 1997).

The most common subtype of type 3 GD is characterzed by the following (Patterson et al., 1993): (i) presentation in infancy or childhood with aggressive systemic disease leading to death in adolescence from complications of portal or pulmonary hypertension; (ii) early development of horizontal supranuclear gaze palsy as the major neurological sign of the illness; and (iii) infrequent seizures, usually as agonal events. Patients presenting with mild sysyemic disease and horizontal supranuclear gaze palsy as the only neurological manifestations have been decribed (Abrahamov et al., 1995; Chabas et al., 1995) and this phenotype is specifically associated with homozygosity for a rare point mutation, D409H (Pasmanik-Chor et al., 1996).

Type 2 and type 3 seperation of GD patient is useful, it has been increasingly clear that the phenotypes of neuronopathic Gaucher represent a continuum from the most severe congenital GD to the mildest form of chorionic neuronopathic Type 3 patients with very mild horizontal supranuclear gaze palsy. GD patients with Type 2 features are able to walk and live well beyond the expected life span. In addition, there are patients with severe systemic disease with isolated supranuclear gaze palsy who developed progressive myoclonic encephalopathy (Schiffman et al., 1997). Although it has been thought that the genotype of these patients plays an important role in determining the natural history of brain involvement (Beutler, 1995a; Mistry, 1995), the extent and mechanism by which a defined genotype leads to a particular

phenotype is not known.

1.7 β -glucosidase

1.7.1 Pathophysiology

1.7.1.1 The storage substance-Glucosylceramide

N-acyl-sphingosyl-1-O- β-D-glucoside is the major natural substrate for acid β-glucosidase. It is also known as glucosylceramide, ceramide β-glucoside, or glucocerebroside, and the enzyme is referred to as glucocerebrosidase, glucosylceramidase, ceramide B-glucosidase, or acid β-glucosidase. Beta-glucosidase (EC 3.2.1.21) is a glucosidase enzyme that acts upon β1->4 bonds linking two glucose or glucose-substituted molecules (i.e., the disaccharide cellobiose). It is an exocellulase with specificity for a variety of beta-D-glycoside substrates. It catalyzes the hydrolysis of terminal non-reducing residues in beta-D-glucosides with release of glucose.

Glucosylceramide is widely distributed in many mammalian tissues in small quantities as a metabolic intermediate in the synthesis and degradation of complex glycosphingolipids, such as the gangliosides or globoside, and as a membrane constituent (Beutler and Grabowski, 1995).

Acid β-glucosidase is a peripheral membrane glycoprotein that composed of 497 amino acids. The acid β-glucosidase amino acid sequence appears to be invariant i.e. there are no functional polymorphisms. The enzyme has been purified from natural and recombinant sources (Beutler and Grabowski, 1995) and is present in lysosomes of all tissues. The enzyme has 5 glycosylation consensus sequences. From that only first 4 are normally occupied. The first glycosylation occupancy appears to be necessary for the co-translational vector folding of the enzyme during synthesis (Berg Fussman et al., 1993). This enzyme has seven cysteine residues and three disulphide bonds in the monomer. Site- directed mutagenesis of each of the cysteines to serine residues indicates that all cysteine, except for C126, are necessary for activity of the enzyme (Grabowski et al., 1997). Site-directed mutagenesis and heterologous expression studies of various mutations identified in GD and other random mutations suggest that residues important to the proteolytic stability of acid β-glucosidase are

present across exons 5 and 6, and also in exons 9 and 10.The carboxy terminal end of the enzyme also appears important to catalytic activity, and the final 10 to 20 amino acids may participate in the formation of lid for the active site similar to other lipases (Ponce et al., 1994; Grabowski et al., 1997). It activity requires the interaction of the enzyme, phospholipids and/or a receptor on the inner lysosomal memebrane, a specific activator protein (saposin C), and an insoluble substrate glucosylceramide.

1.7.1.2 Synthesis

Glucosylceramide is synthesized from ceramide and UDP-glucose by glucosylceramide synthase (Figure 9). The reaction has an optimal pH at 7.8 and Km values of 0.12 and 0.8 mM for ceramide and UDP-glucose, respectively. Both hydroxy and nonhydroxy fatty acylsphingosines serve as substrates. This pathway is present in brain and a variety of tissues and cell types. Although ceramide and sphingosine are synthesized in the ER, glucosylceramide is made in the cis-Golgi complex. A major component of the active site of glucosylceramide synthase is on the cytoplasmic face of the cis-Golgi. It is the only glycosphingolipid synthesized with the polar head on the cytoplasmic surface of the Golgi. Synthesis of the higher order glycosphingolipids from glucosylceramide requires a flip-flop across the Golgi membrane during migration from cis to trans. Alternative synthetic paths occur in neuroblastoma cells via reacylation of glucosylsphingosine and via dolichol-phosphate glucose in BHK-21 cells. It is synthesized by these pathways is destined for the plasma membrane and does not contribute major amounts to the lysosomal degradative pathway (Beutler and Grabowski, 1995).

1.7.1.3 Catabolism

Glucosylceramide is the intermediate in the degradative pathway of most complex glycosphingolipids. Further degradation by the acid β-glucosidase produces ceramide (Figure 1.10), the latter being further degraded by acid ceramidase to sphingosine and fatty acid. Its dispersion has been used to permit endocytosis and absorption of the lipid into the outer leaflet of the plasma membrane. The fate of the internalized glucosylceramide depends on the differentiation state of the cells. In differentiating cells, glucosylceramide is preferentially sorted to the Golgi apparatus following endocytosis, whereas this does not occur in fully differentiaited cells (Beutler and Grabowski, 1995).

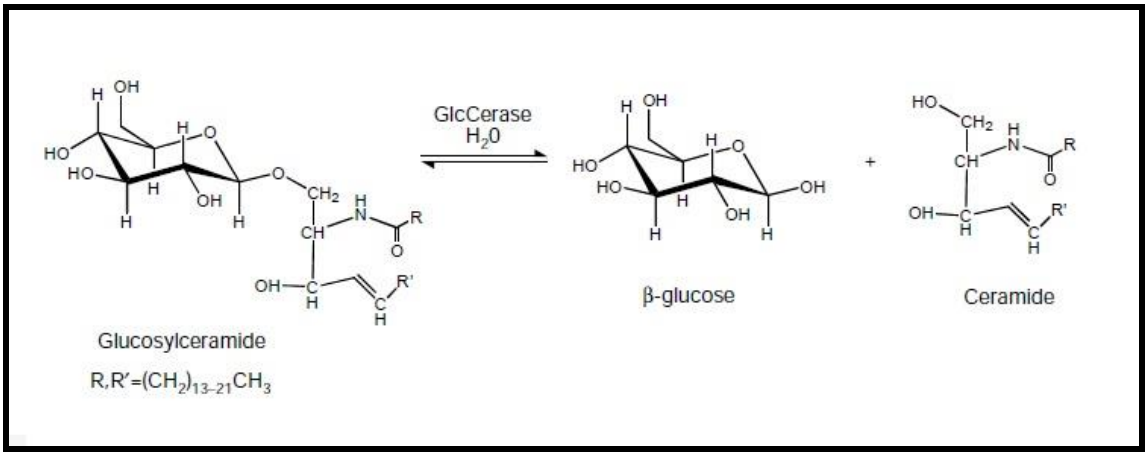

Figure 1.10: Reaction catalysed by acid-β-glucosidase. Acid-β-glucosidase (GlcCerase) hydrolyses the β-glucosyl linkage of glucosylceramide (GlcCer), to yield ceramide and glucose (Dvir, 2003)

1.7.1.4 Tissue and Organ distribution and characteristics

In affected patients, plasma levels of glucosylceramide are elevated 2 to 20 times, but such levels do no correlate with the type of GD. It is associated with lipoproteins. Only the liver, spleen and brains of patients with GD have been subjected to extensive lipid analyses and characterization studies. In liver and spleen there are large (20 to 100) times increase of glucosylceramide. The fatty acid and sphingosyl base compositions of the accumulated glucosylceramides are typical of visceral organs. There is a predominance of c20 to c24 fatty acids and shorter chain sphingosyl bases in all GD variants. Increased levels of glucosylceramide are present in brains from several diseases variant. However, the distribution, amount, and type of this accumulated lipid vary with GD variant (Beutler and Grabowski, 1995).

1.7.2 X-ray structure of β-glucosidase

On the basis of sequence similarity, GlcCerase was classified as a member of glycoside hydrolase family 30, which is a member of the glycoside hydrolase A (GH-A) clan. Inherited defects in GlcCerase result in lysosomal GlcCer accumulation and, as a consequence, GD, the most common LSD (Meikle et al., 1999), which occurs at a frequency of 1 in 40,000 to 1 in 60,000 in the general population, and 1 in 500 to 1 in 1,000 among Ashkenazi Jews (Beutler & Grabowski, 2001; Charrow et al., 2000).

The refined X-ray structure of GlcCerase at 2.0 Å (*R*-factor 19.5%; *R*-free 23.0%)

contains two GlcCerase molecules per asymmetric unit. Its overall fold comprises three domains (Figure 1.11A). Domain I (residues 1–27 and 383–414) consists of one main three-stranded, anti-parallel β-sheet that is flanked by a perpendicular amino-terminal strand and a loop. It contains two disulphide bridges (residues 4–16 and 18–23), which may be required for correct folding (Beutler & Grabowski, 2001). In the crystal structure at residue N19, Glycosylation that is essential for catalytic activity *in vivo* (Berg-Fussman et al., 1993), is seen. Domain II (residues 30–75 and 431–497) consists of two closely associated β-sheets that form an independent domain, which resembles an immunoglobulin (Ig) fold (Orengo et al., 1997; Westheadet al., 1999). Domain III (residues 76–381 and 416–430) is a (β/α) 8 TIM barrel, which contains the catalytic site, consistent with homology to GH-A clan members (Fabrega et al., 2002; Henrissat & Bairoch, 1996). It contains three free cysteines (at positions 126, 248 and 342). Domains II and III seem to be connected by a flexible hinge, whereas domain I tightly interacts with domain III.

Substrate docking shows that only the glucose moiety and the adjacent glycoside bond of GlcCer fit within the active-site pocket (Figure 1.11B), suggesting that the two GlcCer hydrocarbon chains either remain embedded in the lipid bilayer during catalysis or interacts with saposin C. In addition, an annulus of hydrophobic residues surrounds the entrance to the active site (Figure 1.12) and may facilitate interaction of GlcCerase with the lysosomal membrane or with saposin C (Wilkening et al., 1998).

Seven aromatic side chains (F128, W179, Y244, F246, Y313, W381 and F397) line one side of the active-site pocket, and may be involved in substrate recognition, as in other β-glycosidases (Chi et al., 1999; Henrissat & Bairoch, 1993). The commonmutation V394L might perturb this lining, as the bulkier leucine side-chain could cause a conformational change in two residues of the lining, Y244 and F246. Several other mutations (H311R, A341T and C342G; Figure 1.12) occur near the active site and may directly affect catalytic activity. By contrast, two relatively common mutations, R463C and R496H, which cause predisposition to mild disease (Beutler & Grabowski, 2001), are located in the Ig-like domain, at a considerable distance from the active site (Figure 1.11A). L444P, which is mutated relatively frequently to proline or arginine and invariably causes predisposition to severe neuronopathic disease (Beutler & Grabowski, 2001; Erikson et al., 1997), is located in the hydrophobic core of the Ig- like domain (Figure 1.11). Either of the two L444P

mutations might cause a local conformational change by disrupting the hydrophobic core, resulting in altered folding of this domain (Morel et al., 1999). This is consistent with the assumption that these mutations produce unstable proteins (Grace et al., 1994). This suggests an important regulatory or structural function for domain II, perhaps in interacting with saposin C and/or acidic phospholipids. Interestingly, β-hexosaminidase and other family- 20 glycosidases have a similar non-catalytic domain, the function of which is unknown (Mark et al., 2001). The structure of saposin C has recently been determined by nuclear magnetic resonance (NMR) spectroscopy (Protein Data Bank (PDB) ID code 1M12), but its coordinates have not yet been released to the public. However, the structure of its homologue, saposin B (Ahn et al., 2003), shows that the putative active form is a dimer in which a large hydrophobic cavity sequesters the acyl chains ofcerebroside sulphate, and may serve to present it appropriately for hydrolysis by arysulphatase A. It is not yet determined whether such a mechanism would explain the role of saposin C as an activator of GlcCerase, as the limited sequence homology (<14%) between saposins B and C does not allow accurate modelling of the latter. However, the Ig-like domain of GlcCerase may regulate the interaction of GlcCerase with either the lipid bilayer, saposin C or both. Finally, there are no known viable mutations in residues 14–20 of domain I and in the connecting strand (residues 1–10) and loop (residues 21–27), with the exception of the conserved mutation V15L. However, there are seven known mutations in the C-terminal strand of this domain (residues 401–414), including the common severe mutation D409H, which results in unstable protein (Beutler & Grabowski, 2001). This suggests that domain I also has an important regulatory or structural role.

In summary, the GlcCerase structure will allow detailed and systematic analysis of the relationship between disease severity and perturbations in enzyme structure for each of the mutations (Figure 1.12). It will also allow the structure-based design of small molecules that may interact with misfolded GlcCerase and stabilize the structure of some common mutations, such as N370S. The feasibility of the latter approach has recently been shown by use of a chemical chaperone to enhance GlcCerase activity in cultured cells and in *in vitro* assays (Sawkar et al., 2002). Such an approach, together with the mechanistic information that can now be deduced from the GlcCerase structure, paves the way for new and improved therapeutic approaches for treating GD.

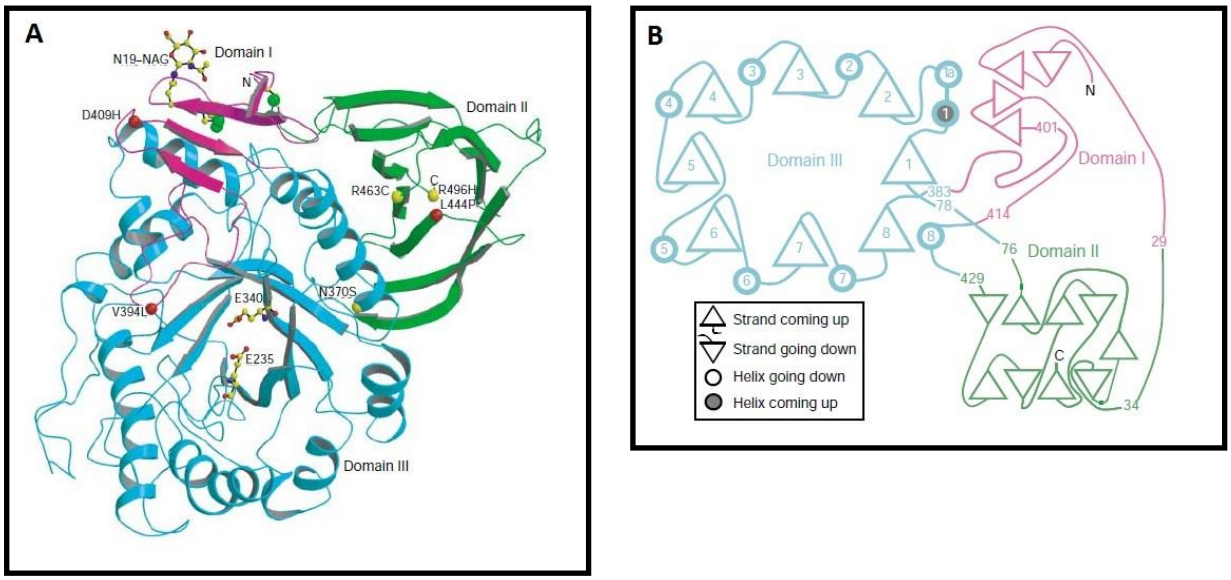

Figure 1.11: The refined X-ray structure of acid-β-glucosidase. (A) Domain I is shown in magenta and contains the two disulphide bridges, the sulphur atoms of which are shown as green balls. The glycosylation site at N19 is shown as a ball-and-stick model. Domain II, which is an immunoglobulin- like domain, is shown in green. The catalytic domain (domain III), which is a TIM barrel, is shown in blue, and the active-site residues E235 and E340 are shown as ball-and-stick models. The six most common acid-β- glucosidase (GlcCerase) mutations are shown as balls, with those that cause predisposition to severe (types 2 and 3) and mild (type 1) disease in red and yellow, respectively. (B) Two-dimensional topology of GlcCerase. The diagram is consistent with a three-dimensional view, looking down the opening of the active-site pocket, as in (A). All connecting loops in the diagram are of an arbitrary length. α-Helices and β-strands of domain III are numbered according to their position in the sequence. For clarity, sequence numbers for certain positions are shown in the connecting loops, and secondary structural elements that consist of four residues or less are not shown. NAG, *N*-acetylglucosamine (Dvir, 2003)

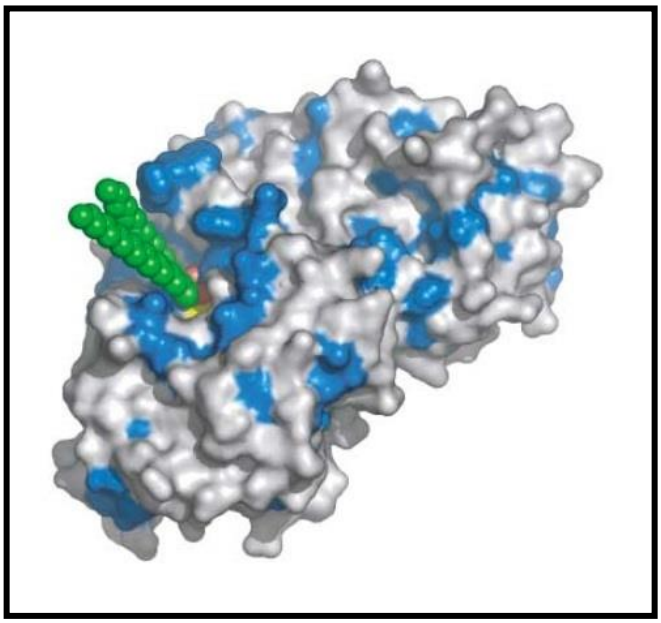

Figure 1.12: The refined X-ray structure of acid-β-glucosidase (created using PyMOL (http://www.pymol.org)), with a model of the docked substrate (based on the coordinates of galactosylceramide (Nyholm et al., 1990) and modified for GlcCer). Hydrophobic residues (W, F, Y, L, I, V, M and C; Hopp & Woods, 1981) are shown in blue, and the active-site residues (E235 and E340) in yellow. GlcCer is shown in CPK format (carbon atoms in green, and oxygen atoms in red) (Dvir, 2003)

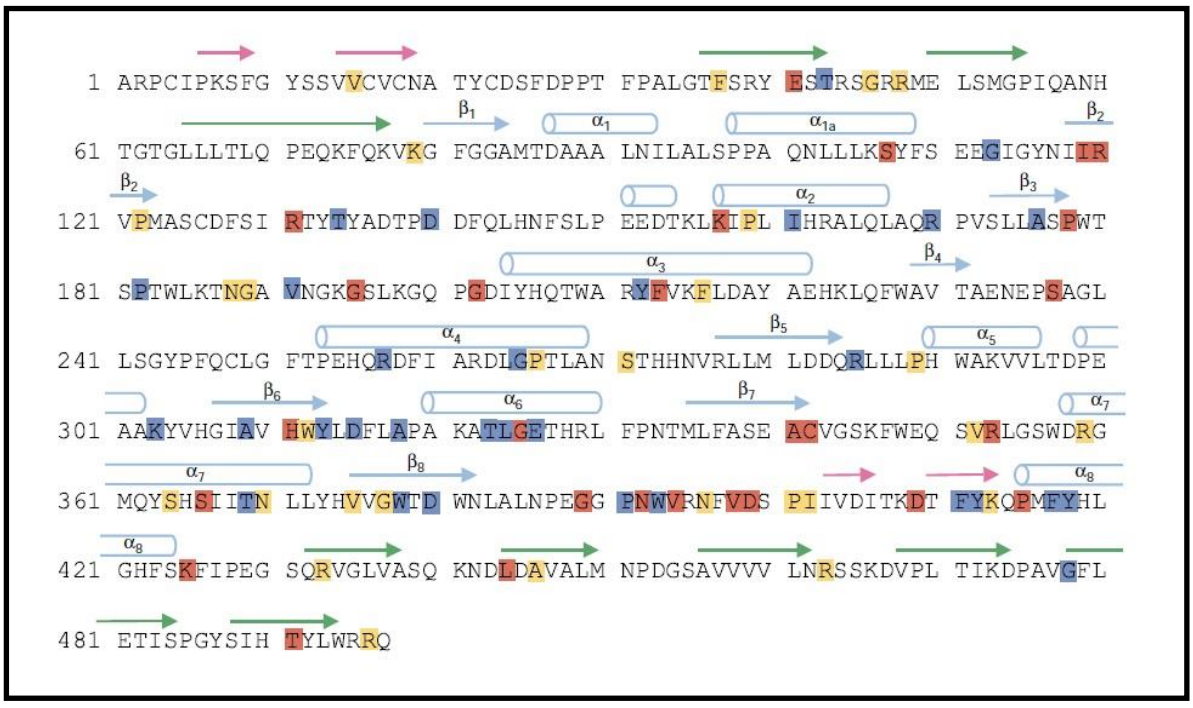

Figure 1.13: **Mutations in acid-β-glucosidase.** (A) The sequence of the 497 residues

of acid-β-glucosidase (GlcCerase). Mutations reported to cause severe disease (http://www.tau.ac.il/~racheli/genedis/gaucher/gaucher.html) is shown in red, those that cause mild disease in yellow, and those for which clinical data documenting severity of the disease are lacking in blue. Only single amino-acid substitutions are included, with frameshifts and splices excluded as the enzyme is not expressed in most of these cases. Helices are indicated by cylinders, and β-strands are indicated by arrows of colours corresponding to those of the domains shown in Figure 1.10 (Dvir, 2003)

1.8 Molecular Biology

1.8.1 Gene and pseudogene structure (*GBA and psGBA*)

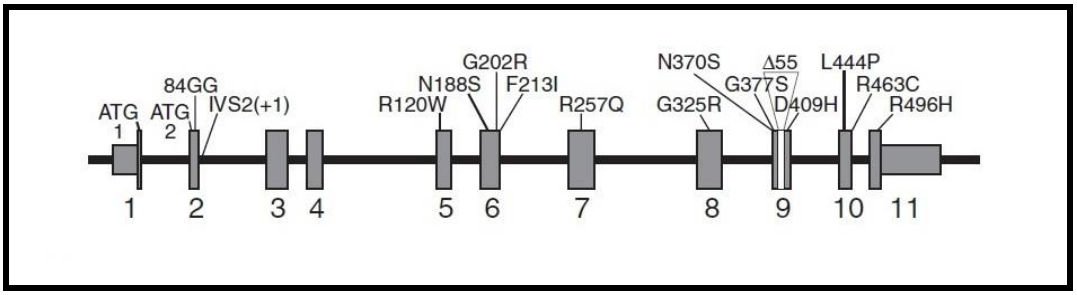

Figure 1.14: *GBA* gene structure(Hruska et al., 2008)

The gene for glucocerebrosidase (*GBA*; MIM) 606463) was localized to 1q21 (Figure 1.14) (Barneveld et al., 1983) and subsequently cloned and sequenced (Ginns et al., 1984; Horowitz et al., 1989; Sorge et al., 1985b). The *GBA* gene comprises of 11 exons and 10 introns, spanning 7.6-kb of sequence. A highly homologous pseudogene (*GBAP*) is located 16 kb downstream (Horowitz et al., 1989) and is 5.7 kb in length, with the same organization of exons and introns as the functional gene (Figure1.1). Despite their different lengths, caused by several Alu insertions in introns of GBA, there is 96% sequence identity between the functional *GBA* gene and *GBAP*. A useful distinction for molecular diagnostic applications is that the pseudogene carries a 55-bp deletion in exon 9. The presence of this conserved pseudogene at the same locus is significant, because recombination events between *GBAP* and *GBA* result in several different GD mutations and groups of mutations (Eyal et al., 1990; Hong et al., 1990; Latham et al., 1990, 1991; Zimran, et al., 1990b).

The region surrounding human glucocerebrosidase has been found to be particularly

gene rich, including seven genes and two pseudogenes within an 85-kb region of chromosome 1q (Figure 1A) (Long et al., 1996; Winfield et al., 1997). The closest downstream gene is metaxin (MTX1), which is transcribed convergently to *GBA* (Long et al., 1996). Metaxin appears to play a role in the preprotein import complex in the outer mitochondrial membrane (Armstrong et al., 1997) and shares a bidirectional promoter with the nearby gene for thrombospondin 3 (Adolph et al., 1995). Metaxin also has a pseudogene, which is located in the 16-kb region between *GBA* and *GBAP*. Both pseudogenes appear to have resulted from a duplication of the region, and are present in primates, but not in other species (Martinez-Arias et al., 2001a; Winfield et al., 1997). The sequences of both *GBA* and MTX1 have been shown through multispecies comparative sequence analyses (Thomas et al., 2003) to have conservation of the exonic regions in humans and nine mammalian species (LaMarca et al., 2004).

1.8.2 mRNA and cDNA structure of *GBA* gene

Acid β-Glucosidase cDNA is about 2.5 kb in length. There are two upstream ATGs that are both utilized in translation. The relative importance of function of this two start site is unknown. The sequence between upstream and downstream ATG is a hydrophilic leader while that between the downstream ATG and the cleavage site of the leader sequence is the typical hydrophobic sequence expected in a leader sequence. It has been suggested that alternatively spliced forms might create one protein product with the hydrophilic and another product with hydrophobic leader sequence, but such splice form have not been identified. mRNAs of several different lengths have been detected probably due to use of alternative polyadenylation sites, alternative splicing or presence of pseudogene mRNA (Beutler and Grabowski, 1995).

1.8.3 Expression of acid β-Glucosidase

The expression levels of acid β -Glucosidase mRNA and activity vary considerably in different tissues (Reiner, 1998a; Reiner and Horowitz, 1988). The 5’ region of the functional gene contains two TATA boxes and two possible CAAT-like boxes (Reiner, 1998a, b; Horowitz, 1989). Using reporter gene constructs, this region from the functional gene is at least 8-10 times more potent in cell lines than the corresponding pseudogene sequences. Detailed studies of exon 1 and 5’ flanking region of the human locus reveal several control regions Figure 1.15. Relative to the

mRNA start sites (+1), the TATA box is located at -28 to -25. Except for about 20-30 bp 5' to the TATA, most of the 5' region is not necessary for full expression in specific cell lines. Interestingly, an orientation, but not positionally, independent transcriptional enhancer was present within exon 1 between +31 and +79. The four transcription factors, OBP (OCT binding protein), AP-1, PEA3 and CBP (CAAT binding protein), had significant promoter activity. The corresponding DNA binding sites were located as follows with reference to the transcriptional start site: OCT (-92 to -99), AP-1 (+70 to +76), PEA3 (near +1) and CBP (-1 to +4) (Moran et al, 1997). There is a TTTAAA consensus binding protein that also participates in the expression of acid β-Glucosidase. The apparent cellular specificity observed in studies will require more physiologiacal verifications in transgenic animals. But the up-regulation of OBP and AP-1 in transformed fibroblasts in concordant with the metabolic needs of the cells for turnover of glucosylceramide, the major GD substrate. Thus, the housekeeping function of acid β-Glucosidase may be concordantly regulated by the transcriptional activation that accompanies cell turnover and proliferation (Zimran, 1997).

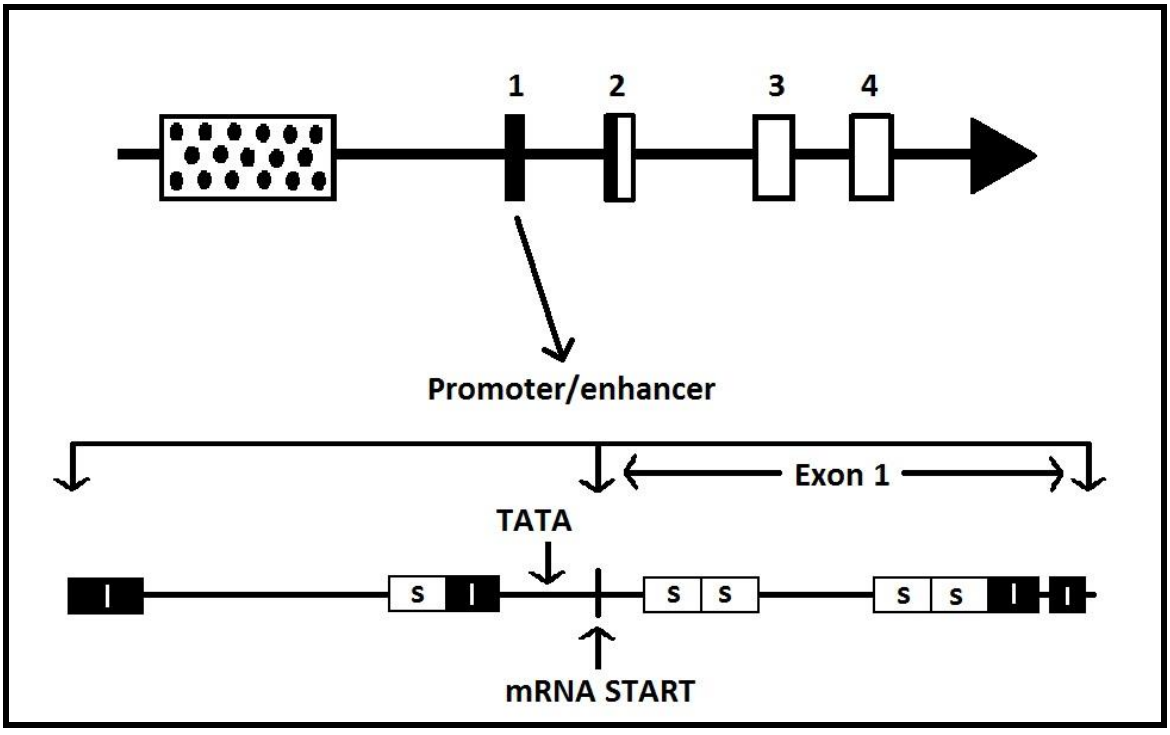

Figure 1.15: An enlarged view of 5' *GBA* region and flanking sequences. The dotted rectangle in the upper panel represents 5' flanking promoter regions. The blackened parts of exon 1 and 2 are untranslated. The region below expands the exon

1 and 5’ flanking region to show in vitro stimulatory (S) and inhibitory (I) sequences (Zimran, 1997)

1.9 Diagnosis

Like other metabolic diseases, LSDs show remarkably varied clinical signs and symptoms, which may occur from the in utero period to late adulthood, depending on the complexity of the storage products and differences in their tissue distribution. Indeed, the recognition of LSD clinical features requires clinical expertise, as most of them are not specific and can be caused by defects in other metabolic pathways (mitochondrial and peroxisomal), or by environmental factors. Even in the presence of typical clinical signs and symptoms, samples and diagnostic tests are different for each group of lysosomal disorders and often are specific to a given disease (Filocamo and Morrone, 2011).

The definitive diagnosis of LSDs therefore requires close collaboration between laboratory specialists and clinicians. For laboratory diagnosis, the clinician must select the appropriate test to be performed on the basis of a comprehensive evaluation that includes not only a physical assessment of the patient but also paraclinical test results (peripheral blood smears, radiological/neurophysiological findings etc) and biomarkers for some of the storage disorders. Additionally, each sample that is sent for testing should be accompanied by a detailed patient case history and family history, to allow the laboratory specialist to make a reliable evaluation of the results that might include indications for other potential investigations.

Before considering specific analyses (enzymatic), preliminary screening tests should be performed. High levels of chitotriosidase can indicate GD and, to a lesser extent, other lipidoses, such as Niemann – Pick, Krabbe (Sheth et al., 2010). All of these preliminary (serum) tests can provide a clue to the enzyme study, and need to be followed up with specific enzymatic analyses performed on suitable samples, leucocytes and/or cell lines (fibroblasts and/or lymphoblasts). Further study of carrier detection need to be carried out by molecular study of the proband and parents.

As about 75 per cent of LSDs are due to a deficiency in lysosomal hydrolase activity, the demonstration of reduced/absent lysosomal hydrolase activity by a specific enzyme assay is an effective and reliable method of diagnosis. In these cases,

molecular analysis can refine the enzymatic diagnosis. The LSDsalso results from non-enzymatic protein defects such as Gangliosidosis GM2, activator defect, MLD, Krabbe, Gaucher requires molecular analysis to be performed on the specific gene for a conclusive diagnosis. Generally, an inherited deficiency of a lysosomal enzyme is associated with LSD. There are, however, individuals who show greatly reduced enzyme activity but remain clinically healthy. This condition, termed as enzymatic 'pseudodeficiency' (Pd), is known in some lysosomal hydrolases. Due to polymorphic genetic variants, it has been reported for lysosomal enzymes such as β- glucosidase (*GBA* gene) arylsulphatase A (*ARSA* gene) (Gieselmann et al., 1989), β-hexosaminidase (*HEXA* gene) (Cao et al., 1997), α-iduronidase (*IDUA* gene) (Aronovich et al., 1996), α-glucosidase (*GAA* gene) (Nishimoto et al.,1988), α-galactosidase (*GLA* gene) (Froissart et al., 2003; Hoffmann et al., 2005), β-galactosidase (*GLB1* gene) (Gort et al., 2007), α-fucosidase (*FUCA1* gene) (Wauters et al., 1992) and β-glucuronidase (*GUSB* gene) (Chabas et al., 1991; Vervoort et al., 1995).

Conversely, there are circumstances in which affected individuals with a clinical/paraclinical picture resembling some glycosphingolipidoses show normal activity of the relevant lysosomal enzyme. These patients should be investigated for a potential defect of an activator protein involved in glycosphingolipid breakdown.

GD can result not only from a deficiency of an enzymatic activity, but also from defects of sphingolipid activator proteins or saposins. In these cases, conclusive diagnosis requires a comprehensive evaluation based on a range of diagnostic procedures, including neuroradiological, neurophysiological, biochemical/enzymatic and molecular tests.

1.9.1 Clinical recognition

GD clinical recognition depends on the sub type of the diseaseinvolved. Patients with Type-I (non-neuronopathic) are diagnosed most often because of organomegaly, haematological abnormalities (such as anaemia and thrombocytopenia), or bone disease. Due to extreme variability in disease severity, age at diagnosis can vary from infancy to old age. Type-II (acute neuronopathic) and Type-III (subacute neuronopathic patients) are clinically recognised by both the characteristics neurological signs and organomegaly. Type-2 patients are diagnosed in infancy and

Type-3 usually in early childhood, but diagnosis may be delayed to early adolescence (Zimran, 1997).

1.9.2 Laboratory Diagnosis

Laboratory diagnosis of all types of GD isbased on primarilyenzymatic testing and DNA analysis of mutations in the glucocerebrosidase gene. Historically, the histological diagnosis isbased on the presence of the 'Gaucher cell', a characteristic lipid-laden cell of the monocyte-macrophage lineage. This cell however isnot specific for GD and its demonstration requires invasive testing (biopsy of the bone marrow or other organs). Since enzymatic and DNA testing are both highly specific and require only a simple blood collection whereas histological diagnosis no longer has a role in confirming a clinical suspicion of GD (Beutler and Saven, 1990).

1.9.3 Screening test with plasma chitotriosidase

Chitin, the linear polymer of 1, 4-linkedN-acetylglucosamine, is a structural component of cell walls and coatingsof many organisms. For a long time it was thought that mammals are unable to produce endoglucosaminidases that fragment chitin. Investigations on GD led to the serendipitous discovery of a functional endogenous chitinase in man (Hollak et al., 1994; Renkema, 1995 et al.; Boot et al., 1995). It was initially detected in man by its capability to hydrolyze chitotrioside substrates and was named chitotriosidase (Hollak et al., 1994). It has been observed that serum samples from Gaucher patients show a 1000-fold elevated capacity to hydrolyze 4-methylumbelliferyl-D-chitotriose (Aguilera et al., 2003). The responsible enzyme, named chitotriosidase, isshown to be able to cleave naturalchitin and a variety of artificial chitin-like substrates such as4-methylumbelliferyl and p-nitrophenyl chitooligosaccharides. Chitotriosidase belongs to the family 18 of glycosylhydrolasesand is highly homologous to chitinases from lower organisms. The enzyme consists of N-terminal TIM-barrel structure containing the catalytic groove and a C-terminal chitin-bindingdomain connected by a short hinge region. The crystal structure of the 39-kDa catalytic domain and its complexes with a chito oligosaccharide and allosamidin has been described (Fusetti et al., 2002).

The CHIT1 gene is localized in the chromosome 1q31- q32 (Eiberg and Den Tandt, 1997), and is composed of 12 exons spanning about 20 kb of genomic DNA (Fusetti et

al., 2002). A recessive inherited mutation of CHIT1 gene, which consists of 24-bp duplication in exon 10 that subsequently activates a cryptic 39-splice site in the same exon, generates a spliced form of mRNA (Malaguarnera, 2006). The spliced mRNA encodes an enzymatically inactive CHIT1 protein that completely lacks the 29 amino acids in an internal stretch and this particular mutation is the predominant cause of CHIT1 deficiency (Boot et al., 1998).

Chitotriosidase (CHIT1 or chitinase-1) is selectively expressed in chronically activated tissue macrophages, like the lipid-laden storage cells that accumulate in large quantities in various tissues of Gaucher patients (Aerts and Hollak, 1997). It is actively produced by activated Kupffer cells, resident macrophages of the liver, which activate hepatic stellate cells to synthesize collagen, and the overproduction of collagen subsequently induces hepatic fibrosis and liver cirrhosis (Malaguarnera, 2006). Tissue macrophages largely secrete newlysynthesized 50-kDa chitotriosidase, but about one-third is directly routed to lysosomes and proteolytically processed to the39-kDa unit that remains catalytically active (Renkema et al., 1997). Intriguingly, one in every three individuals from various ethnic groups carries one abnormal chitotriosidase gene with a 24-bp duplicationthat prevents production of enzyme (Boot et al., 1998). About 6% of the population is homozygous for this mutant allele and consequently completely lacks chitotriosidase activity.

In a healthy population, CHIT1 activity is very low and originates in the circulating polymorphonuclear cells. In patients with GD, the activity of this enzyme is elevated 10 – 1000 fold in blood (Hollak et al., 1994; Malaguarnera, 2006; Aerts et al., 1999; Guo et al., 1995; Bouzas et al., 2003; Baroe et al., 2007; Wajner et al., 2007; Sheth et al 2010). However, CHIT1 deficiency and carrier frequency occur in 6% and 30- 40% of the general population due to mutations, respectively (Staretz-Chacham et al., 2009; Novakovic et al., 2010). This is also a valuable diagnostic biomarker for monitoring the enzyme replacement therapy (ERT) in GD (Hollak et al., 1994). The decrease in CHIT1 activity after ERT may cause an alteration in tissue macrophage activation rather than a reduction of Gaucher cells in this disease. CHIT1 activity has the sharpest decline during the first year of ERT. In general, ERT had the highest efficacy during the first six months and after the first year, during which CHIT1 had the highest reduction in its enzymatic activity. This suggests that the rapid initial reduction in CHIT1 activity during ERT may be due to the alteration of either

activation or differentiation of macrophages and their precursors, rather than a decrease in Gaucher cell burden (Harmanci and Bayraktar, 2008; Brady et al., 1994).

1.9.4 Biochemical Genetic Testing (BGT)

Biochemical genetic testing (BGT), including the assay of enzymatic proteins, is feasible for most LSDs and is the gold standardfor the diagnosis of primary lysosomal enzyme deficiency.

Lysosomal enzymes are present in almost all tissues and biological samples. The choice of the sample type to be analysed is based on (i) the level of an enzyme's activity in a specific tissue, (ii) the sample stability during its transfer to the referring laboratory and (iii) the time of diagnosis (Filocamo and Morrone, 2011).

Although enzyme activity can be assayed in some biological fluids, such as plasma, serum and urine, several enzymatic Pds(Pseudodeficiencies) have been reported in serum or plasma, so their use can lead to pitfalls in diagnosis (Hoffmann et al., 2005; Thomas, 1994). Leucocytes are the mostappropriate biological samples, although possible interference betweenisoenzymes, enzyme degradation, reference enzymes study should be taken into consideration. Fibroblast samples represent the gold standard in diagnosis, since they express the optimum enzyme activity; however, they require an invasive skin biopsy and culturing. Epstein–Barr virus- transformed B-lymphoblast culture obtained from a non-invasive blood sampling can be useful, bearing in mind, however, that lymphoblasts do not express some enzymatic activities, such as arylsulphatase A. Lysosomal enzyme assays are usually performed using synthetic (fluorimetric or colorimetric) substrates showing undetectable or very low enzyme activity in the leucocytes or plasma of an affected individuals.

The complete absence of lysosomal enzyme activity generally confirms diagnosis. Conversely, the presence of normal lysosomal enzyme activity cannot exclude a specific diagnosis if it is accompanied by suggestive clinical symptoms and/ or the abnormal presence of metabolites in the urine and/or storage in peripheral smear and/or tissue biopsy. For example, a patient who presents with a clinical profile resembling GD, with high levels of chitotriosidase activity and increased concentrations of glucosylceramide in plasma and normal β-glucosidase activity in skin fibroblasts, should be referred for a molecular genetic study of the prosaposin

gene (PSAP), which codes for the cofactor Sap C required for the function of β-glucosidase (Tylki-Szyman et al., 2007).

Peripheral blood leucocytes normally contain abundant acid β-glucosidase activity, and their capacity to cleave glucosylceramide is markedly diminshed in GD. Under proper assay conditions synthetic, water–soluble substrates for β-glucosidase are very useful in the diagnosis of GD (Beutler, 1995). Low glucocerebrosidase activity (<10% of normal) in leukocytes or fibroblasts is the diagnostic standard of GD, and enzymatic testing should performed in all cases. Its major advantages are that it is used for diagnosis in affected patients and it is not depend on ethnic origin or previous knowledge of the specific familial mutation. The limitations of enzymatic testing are labile natureof enzyme activity, the lack of correlation between in vitro enzymatic activity and the type and severity of the disease, and poor differentiation between heterozygote carriers and normal (Zimran, 1997).

The detection of residual lysosomal enzyme activity should be carefully evaluated, together with clinical and instrumental findings. Molecular genetic testing (MGT) can reveal polymorphisms that potentially lead to an enzymatic Pd (Pseudodeficiency)(Filocamo and Morrone, 2011).

1.9.5 Molecular Genetic Testing (MGT)

MGT performed on DNA comprises a range of different molecular approaches for investigating the entire gene-coding regions and exon– intron boundaries, as well as 50- and 30-untranslated regions (UTRs). It can confirm the enzymatic diagnosis of LSD, and is essential for the carrier study of LSDs resulting from non- enzymatic lysosomal proteins and in post-mortem diagnoses when the only suitable specimens available are DNA samples. MGT can also contribute to elucidating the findings of high biochemical residual enzyme activity in affected patients and very low enzyme activities in unaffected patients with pseudodeficiency in MLD especially (Gieselmann et al, 1989; Cao, 1997 et al; Aronovich et al, 1996; Nishimoto et al., 1988; Froissart et al., 2003; Hoffmann et al., 2005; Gort et al., 2007; Wauters et al., 1992; Chabaset al., 1991; Vervoort et al., 1995). Moreover, it is useful in genotype – phenotype correlation studies for some diseases and for identifying at-risk family members.

MGT can clarify the type of genetic variation and its impact on the protein and on the presence of residual enzyme activity. This information is crucial in evaluating treatment options, such as enzyme replacement therapy (ERT), to date only available for some disorders, and alternative treatments such as or substrate reduction therapy (SRT) (Mistry, 2015 et al; Cox et al., 2015) and pharmacological chaperones therapy (PCT) (Zimran et al., 2013; Yang et al., 2013).

Particular care should be taken when interpreting genotype–phenotype correlations, even in the context of a recurrent mutation, as some patients carrying the same lesion may present with different clinical phenotypes, suggesting that other factors, such as polymorphic variants, genetic modifiers or RNA editing-like mechanisms (Lualdi et al., 2010), can lead to changes in protein function which could influence the clinical phenotype.

DNA based diagnosishas major disadvantage over enzymatic diagnosis of GD in that the results may be normal in cases with deep intronic variations, presence of complex allele(functional and pseudo gene). Thus, even when DNA is examined for many different known mutations, a normal result does not ensure the absence of a GD allele(Beutler and Grabowski, 1995). Since Gaucher's disease is recessive, patients are expected to have two mutated alleles: either the same mutation on both alleles (mutation homozygotes) or different mutation on each allele (compound heterozygotes). Theoretically, the entire glucocerebrosidase gene could be sequenced in each patient: the entire glucocerebrosidase cDNA (coding sequence) is 2.5 kb in size, and genomic sequencing of approximately 7 kb would also allow identification of intronic mutations resulting in splicing aberrations (Beutler and Grabowski, 1995). In practice, DNA analysis is usually confined to rapid testing of limited number of known mutations, utilizing PCR-based techniques. Thus, when compared with the complete (100%) sensitivity of enzymatic testing, the actual sensitivity of DNA analysis depends on the scope of mutations tested, and the combined frequency of these mutations in patients from a similar ethnic background. In contrast to enzymatic testing, which does not predict disease type or severity, DNA testing allows some prediction of disease course, based on observed genotype-phenotype correlations. Thus, laboratory diagnosis of GD should be based on enzymatic testing, and refined using DNA analysis (Zimran, 1997).

1.9.6 Dried Blood spot (DBS)

Dried blood spots (DBS) on filter paper for measurement of lysosomal enzymes activities was introduced in 2001 (Chamoles, 2001) as a method with several advantages over testing in leukocytes/fibroblasts. First, it requires only a few drops of blood. Second, the main benefit is the possibility to mail the DBS samples to specialized laboratories, making it possible to send samples to other cities, regions or countries for testing. Finally, this method allows microplate adaptation, permitting the simultaneously measurement of multiple samples and automation. Potentially, it could also be applicable to newborn screening.

Due to different techniques of blood sample collection that could change the enzyme activity, specific lab recommendations for collection should be followed (Olivova et al., 2009), in terms of the specific filter paper and use or not of anticoagulant. The usefulness of DBS for LSD diagnosis is not universally accepted by medical community. Generally, physicians recommend assaying enzymatic activity in leukocytes/fibroblasts in order to confirm an abnormal result in DBS (Chamoles et al., 2009).

1.9.7 Prenatal testing

Both enzymatic and molecular testing described above can be performed prenatally, either by chorionic villus sampling (CVS) at 8-10 weeks of pregnancy, or by amniocentesis at 15-17 weeks of pregnancy. In addition, diagnosis on material obtained by CVS may be complicated by maternal contamination in up to perhaps 6% of the cases (Teshima et al, 1992; Smidt- Jensen et al., 1993; Association of Clinical Cytogeneticists Working Party, 1994). Molecular and enzymatic testing is important to be correlated in these types of cases. Recent studies in India have shown that CVS and AF fibroblasts can be used with high sensitivity and specificity (Sheth et al., 2015, Verma, 2016)

1.9.8 Diagnosis of carriers

GD carriers have sufficient glucocerebrosidase activity and are completely healthy in this regard. Thus, determination of carrier status is purely a laboratory diagnosis, and is important only for its reproductive implications. In contrast to its usefulness in diagnosing affected subjects, enzymatic testing does not reliably distinguish between

carriers and non-carriers. Although carriers have, on average, half–normal glucocerebrosidase activity in leukocytes and fibroblasts, there is a considerable overlap with non-carriers. In contrast DNA testing is unequivocal for those mutations tested, so in families with known mutations, DNA testing identifies carriers and non-carriers with complete accuracy. In families where one or both mutations are unknown, carrier detection must rely on enzymatic testing, which may result in misclassification of some carriers as non-carriers, or on identification of the familial mutation by complete sequencing of the glucocerebrosidase gene (Zimran, 1997). Carrier status can also be determined indirectly using polymorphisms in the glucocerebrosidase gene and the tightly linked pyruvate kinase gene (Glen et al., 1994). A number of highly polymorphic markers in this region have recently been described (Sidransky et al., 1997), so most families in which parents and an affected child are available are likely to be informative.

1.10 Recurrence Risk

GD is autosomal recessive inherited disorder. Therefore in this disease there is no risk for an affected child unless both parents are carriers. At risk couples, i.e. couples where both partners are carriers, have a 25% risk for an affected child in each pregnancy. Many at-risk couples are identified only after the birth of an affected child. Healthy children of at-risk couples have atwo-third chance of being carriers, and a one-third chance of being non-carriers. These a priori risks should be taken into account in families where molecular diagnosis of carrier status is not possible (Zimran, 1997).

1.11 Tissue Abnormalities

The abnormal accumulation of storage cells in tissues of Gaucher patients, and the associated fibronectin lesions and splenic fibrovascular nodules (Lee, 1996), may result in marked changes in biochemical tissue composition. Most striking observations are10-1000 fold increased in glucosylceramide concentration in spleen from GD patients (Suzuki, 1982; Barranger and Gins, 1989). Markedly elevated glucosylceramide concentrations have also been observed inthe bone marrow (Suzuki, 1982) and liver (Barranger and Gins, 1989), as well as brain (Svennerholm et al., 1982) of neuronopathic Gaucher patients.

1.12 Treatment

1.12.1 Enzyme replacement therapy (ERT)

Enzyme therapy (ET) for GD was FDA approved in 1991 (Ceredase®, alglucerase; Genzyme Corporation, Cambridge, MA, USA). Because of the clear limit on the amount of this human placental GCase and potentials for bio-contaminants, recombinant human GCase, imiglucerase (Cerezyme®, imiglucerase, Genzyme Corporation, Cambridge, MA, USA) was developed and was FDA approved in 1994 and subsequently in other countries. Imiglucerase is produced in bioreactors that culture Chinese hamster ovary (CHO) cells expressing human GCase. Imiglucerase differs from alglucerase by having an arginine to histidine substitution at amino acid residue 495. The oligosaccharide structures are very similar. Over the past 15 years, ET with imiglucerase has become the standard of care for treatment of significantly symptomatic GD type 1, and safety and efficacy data and dose response characteristics are available on 5,000 such patients (Andersson et al., 2008; Weinreb et al., 2002).

VPRIV® (velaglucerase alfa, Shire Human Genetic Therapies, Inc, Cambridge, MA, USA) was FDA approved in 2010 for ET in GD patients by the FDA and European Medicines Agency. Velaglucerase alfa is produced in a human fibrosarcoma cell line using Gene-Activation® technology, has different N-glycosylation structures than imiglucerase, and has the amino acid sequence of placental human GCase (Brumhstein et al., 2010). Review of published clinical trials for velaglucerase alfa demonstrates that it is safe and efficacious in the treatment of GD. Furthermore, studies indicate that velaglucerase alfa and imiglucerase are clinically equivalent with regards to hematologic, hepatic, and splenic manifestations (Zimran et al., 2010; 2007).

Uplyso® (Taliglucerase alfa, prGCD, Pfizer Inc, New York, NY, USA) is in clinical trials. Taliglucerase alfa is a human GCase produced in carrot cells and contains the same arginine to histidine substitution at amino acid residue 495 as imiglucerase. In addition, taliglucerase also contains additional N- and COOH- terminal non-natural amino acids (Shaaltiel et al., 2007) and specific plant-derived -xylose and -fucose in the oligosaccharide structures. As of this writing, detailed results of clinical trials of

taliglucerase alfa have not been released.

Despite differences in cell culture/manufacturing process, glycosylation patterns, and amino acid structure among the preparations, these differences do not appear to significantly affect their clinical properties, although data are limited for taliglucerase alfa. Recent results suggest that the differential glycosylation patterns and amino acid structures affect antibody conversion rates in patients. A smaller (1%) IgG conversion rate for velaglucerase alfa was reported compared to 13%–15% in those treated with imiglucerase (Ruiz et al., 2010).

Despite the immense success of ET, it has certain disadvantages. ET requires intravenous access for infusions over a few hours every two weeks. Many patients find this to be very intrusive. Availability/maintenance of adequate peripheral or central venous access is also challenging, and the costs for supplies and skilled nursing (in addition to the considerable cost for the drug itself) to maintain the access are significant. Unfortunately, many patients, particularly those in developing countries, do not have access to the specialized care required to provide ET. Recent production and manufacturing issues with imiglucerase have highlighted the challenges of partially treating the disease during medication shortages. Indeed, during this tumultuous period, many experienced objective (i.e, decreased platelets, hemoglobin) and subjective (ie, fatigue, bone pain) worsening of their disease. Furthermore, certain tissues, ie, lung, and lymph nodes, have been found to be poorly accessible to the recombinant enzyme, resulting in diminished/absent therapeutic response in these tissues (Burrow et al., 2007). Importantly, ET does not have an impact on the neurological manifestations of neuronopathic GD since the enzyme does not enter the CNS in therapeutic levels (Burrow et al., 2011).

1.12.2 Substrate reduction therapy (SRT)

Oral therapies have been explored using small molecules. Norman Radin first proposed the concept of substrate reduction therapy as a potential therapeutic option for GD in 1976 (Radin, 1976). Since that time, two different preparations, Miglustat® (N-butyldeoxynojirimycin; Zavesca®, Actelion Pharmaceuticals, Allschwil, Switzerland) and Eligustat tartrate (Eliglustat®, Genzyme Corp, Cambridge, MA, USA) have been developed and are either licensed for use in GD type 1 (miglustat) or in clinical trials (eliglustat tartrate).

The imino sugar N-butyl deoxynojirimycin, miglustat, an inhibitor of glucocerebroside synthase, the first committed step in glycolipid biosynthesis, was a harbinger of oral substrate inhibitors for GD (Radin, 1976). Although clinical trials showed significant effects on key disease parameters (Cox et al., 2000; Heitner et al., 2002; Eistein et al., 2004), the problematic safety profile led to a relatively narrow label indication when miglustat (Zavesca; Actelion) was approved by the European Medicines Agency for patients with mild-to-moderate GD who are unsuitable for ERT (2002) and by the FDA for patients in whom ERT is not a therapeutic option (2003). Nevertheless, with no other modalities capable of affecting neurologic features, this SRT has the potential to cross the blood-brain barrier and is viewed as a prototype for therapeutic management of neuronopathic forms. Moreover, the drug is oral, obviating many of the inconveniences of intravenous ERT. Unfortunately, the clinical trial with miglustat in type III GD failed to achieve neurologic benefits (Schiffman et al., 2008) because of its inferior efficacy in patients with type I GD (compared with ERT) combined with a higher prevalence of side effects (Hollak et al., 2009).

Eligustat tartrate is a specific, competitive glucosylceramide synthase inhibitor that has a similarity to the ceramide component of glucosylceramide. It is a ceramide analog of the substrate (unlike the glucose moiety as in miglustat) with a better safety profile and higher potency than miglustat. The 2-year results of the phase 2 trials have shown dramatic improvement in key clinical parameters in 20 of 24 patients with type I GD (Lukina et al., 2010). Although this oral SRT will probably achieve market approval (pending satisfactory safety data), it will require long-term experience (longer than for the new enzymes) because of its complex cytochrome P450 metabolism that complicates the use of some medications (McEachern et al., 2007) and because of potential nontrivial cardiotoxicity. Importantly, eliglustat does not penetrate the blood-brain barrier and hence has no added value for type III GD.

1.12.3 Pharmacologic chaperone therapies (PCT)

Lysosomal enzymes may undergo misfolding as a result of mutations in the encoding gene. Misfolded proteins are capable of aggregation and accumulation in cells; this may lead to cell death. They are therefore normally eliminated by the endoplasmic reticulum-associated degradation pathway (ERAD) with the help of naturally occurring molecular ‘chaperones’, small molecules that ensure their safe degradation

via this pathway (Ellgaard & Helenius, 2001; Jarosch et al., 2003). In some cases, the active site may fold normally, which means that they are capable of hydrolysis. Such proteins are ideal candidates for salvage by pharmacological 'chaperones'. These are specific, small molecular weight ligands that reversibly bind to such proteins, stabilize them and ensure their correct targeting to the lysosome. An essential prerequisite is that the binding is reversible. That is to say, having safely 'chaperoned' the mutant enzyme to the lysosome, the ligand– protein complex must then dissociate so that the enzyme is free to bind to its substrate. This approach is also known as enzyme enhancement therapy and is attracting considerable interest, especially as it has been shown that chaperones are capable of crossing the BBB and may therefore have therapeutic potential for the CNS. This approach is especially applicable in GD because only a modest increase in residual glucocerebrosidase should be sufficient to ameliorate the phenotype. Moreover, these small molecules should be able to cross the blood-brain barrier (Vellodi, 2005).

The first PCT in clinical trial used isofagomine tartrate (Amicus Therapeutics) (Kornhaber et al., 2008), but phase 2 trials failed to meet endpoints, and further development was abrogated.

A second PC is ambroxol hydrochloride (ExSAR Corporation) (Maegawa et al., 2009), originally developed as a mucolytic agent 30 years ago (Mucosolvan; Boehringer-Ingelheim), and available over the counter in many countries. Ambroxol has also been used for treatment or prophylaxis or both of neonatal respiratory distress syndrome (Laoag-Fernandez et al., 2000). Ambroxol off-label administered to 12 mildly affected patients with type I GD in 2009 with only the 2 thinnest patients having positive results, suggesting the need for higher doses. Hence, formal clinical trials, using higher doses, are necessary before ambroxol can be considered for its potential to benefit type III GD. All these trials suggets, PCs as the aceptableoption in GD with subacute and acute neuronopathic form in addition to ERT(Zimran, 2011).

1.12.4 Splenectomy

Splenectomy has long been the treatment of choice to avoid severe hypersplenism or to relieve compression of neighbouring organs (Zimran, 1997). It is a very effective treatment for the thrombocytopenia and to a considerable extent for the anemia that often occurs in the course of GD(Beutler and Grabowski, 1995). It is also indicated

when splenomegaly is so massive as to become symptomatic and to interfere with normal growth and development. While the initial response to splenectomy is usually satisfactory, concern has been expressed about the possible effect of the removal of the spleen on progressive deposition of glycolipid in other organs (Beutler and Grabowski, 1995). It seems reasonable that removing an organ that serves as an important storage site would result in an accelerated deposition in other organ such as a skeleton (Beutler and Grabowski, 1995) and, in Type-III disease, the central nervous system (Beutler and Grabowski, 1995). The evidence that this is the case is largely anecdotal. In Norbottnian patients it has been found to accumulate larger amounts of glucosylceramide in the plasma and brains following splenectomy, which correlated with a more rapid clinical deterioration (Beutler and Grabowski, 1995). In this group of patient's ataxia, spasticity and intellectual regression were only seen among splenctomized patients (Erikson, 1986). However, since the need for splenectomy in these patients may simply reflect a more severe phenotype, the role of splenectomy in the pathogenesis of brain involvement in the GD remains unclear. It is recommended that conservative management and that splenectomy be reserved for patients with severe thrombocytopenia, growth retardation and/or mechanical cardiopulmonary compromise. Partial splenectomy was introduced in an attempt to obtain the therapeutics benefits of splenectomy while avoiding the possible adverse effect on the course of the disease (Beutler and Grabowski, 1995). The procedure was also proposed as a means of avoiding susceptibility to sepsis that occasionally follows to splenectomy (Beutler and Grabowski, 1995). Since there is some regrowth of the splenic remnant (Beutler and Grabowski, 1995), it is indeed possible that allowing some of the spleen to remain could prevent progression of the disease. Partial splenectomy may decrease the risk of severe bacterial infections. However, it requires greater surgical expertise and may not prevent subsequent bone complications. At present, splenectomy is indicated as an adjunct to ERT, in patients in whom the enlarged spleen causes a compression syndrome or severe hypersplenism and who do not respond to the high doses of ERT (Zimran, 1997).

1.12.5 Bone marrow transplantation

Children with GD Type-III have been treated with allogenic bone marrow transplantation (Hobbs et al., 1987; Ringden et al., 1995). The haematological and visceral results were excellent, e.g. the childrens body growth normalized and their

general well-being improved. Additionally, no further neurological or mental deterioration were noticed in Norbottnian patients. However, this group of patients is relatively small and definite conclusions concerning neurological effect cannot be made. Furthermore, the significant morbidity and mortality of bone marrow transplantation limits its use. The effect of bone marrow transplantation on patients with progressive myoclonic epilepsy is not known. Bone marrow transplantation in Type-II patients has not, to our knowledge been attempted.

The type-I GD phenotype is expressed entirely as aresult of change in macrophages; progeny of the hematopoietic stem cell. It was therefore logical that allogenic marrow transplantation can be done. The first patient with GD to undergo transplantation showed clearance of Gaucher cells from the marrow in about 6 months (Beutler and Grabowski, 1995) but died of infection before 1 year.Subsequently, a number of other patients with type 1 GD (Beutler and Grabowski, 1995)have undergone transplantation. Although the response to transplantation has been favorable in surviving patients, there have been other deaths secondary to the transplantation procedure, which is still a high-risk form of treatment. Transplantation has also been carried out in patients with type 3 disease(Beutler and Grabowski, 1995). The indications for marrow transplantation are uncertain. Transplantation costs less than enzyme augmentation. Moreover, it provides a permanent cure when successful. However, it is difficult to recommend transplantation of patients with type 1 disease under most circumstances because of 10 percent mortality following transplantation, even under the best circumstances, and because of adverse long-term effects on growth and development (Beutler and Grabowski, 1995). The risk is even higher in patients with advanced organ dysfunction, the very patients who most need treatment. Transplantation seems to be more appropriate treatment method for type 3 disease, particularly since it is unknown if the neurologic disease, could be prevented or arrested by the administration of an enzyme that does not cross the blood-brain barrier.

1.12.6 Gene Transfer

GD can be cured by replacing the patient's defective hematopoietic stem cell with a genetically normal stem cell from another individual, correction of the defect in the patients own hematopoietic stem cells should be an effective way to treat this disease.

However, since stem cells that produce acid β-glucosidase would not have a proliferative advantage over those that do not, cure would be expected only if the patient's untransformed cells were at least partially ablated by chemotherapy or irradiation. Thus, a rational strategy for the treatment of GD would be marrow ablation followed by autologous transplantation with transformed hematopoietic stem cells. Considerable effort has been expended to develop the required efficient gene transfer technology(Beutler and Grabowski, 1995).

The transfer of stable, functional acid β-glucosidase into cultured fibroblasts and transformed lymphoblasts using a retroviral vector has been readily accomplished (Beutler and Grabowski, 1995)but transfer into hematopoietic stem cells is much more difficult(Beutler and Grabowski, 1995). Relatively high efficiency transfer of the human acid β-glucosidase cDNA into murine and human hematopoietic stem cells or progenitors has been accomplished (Beutler and Grabowski, 1995) with evidence of sustained long-term expression of β-glucosidase in mice that have undergone transplantation (Beutler and Grabowski, 1995).

1.12.7 Orthopaedic procedures

The quality of life of patients with GD type I may be greatly enhanced by appropriate orthopedic surgical intervention. Hip replacement has been particularly useful (Beutler and Grabowski, 1995)and successful replacement of knee joints has also been accomplished. Although prosthetic shoulder and knee replacements have been highly successful, care should be taken to choose the correct prosthesis length and anchoring technique for each patient depending on the extent of the bony abnormalities in the affected limb. In patients with very extensive thinning of cortical bone, joint replacement may not be practical because of the lack of sufficient bone structure to support the prosthesis. Furthermore joint replacement in children is not desirable because it interferes with limb growth, and the prosthesis has a relatively short life.