1.1 INTRODUCTION

1.1.1 Lysosomal storage disorders: A brief overview

The lysosome was introduced by De Duve and his colleagues in 1955 to indicate a membrane-surrounded cytoplasmic particle containing acid hydrolases that was isolated by centrifugal cell fractionation. Afterwards, the lysosomes were cytochemically confirmed by Novikoff in 1961 using a suitable staining reaction for acid phosphatase and some other acid hydrolases. This was the scientific discovery that would lead to the understanding of the physiological basis of the lysosomal storage disorders (LSDs). LSDs are known from the nineteenth century with appearance of first medical reports of the patients suffering from genetic and metabolic disorders. Disease like Tay-Sachs disease (TSD) was reported in 1881 (Tay, 1881; Sachs, 1887), Gaucher disease in 1882 (Gaucher, 1882) and Fabry disease in 1898 (Fabry, 1898). In 1963, Pompe disease (Glycogen storage disorder type II or Glycogenesis type II; OMIM#232300) was the first to be identified due to deficiency of α-glucosidase (Hers, 1963). Subsequently, the clarification of several enzyme defects led to the initial categorization of the various LSDs according to their clinical pictures, pathological manifestations and the biochemical nature of the un-degraded substrates (Dingle and Fell 1969-1975). Although part of this categorization is still maintained, it is continually simplified on the basis of newly acquired knowledge on the underlying molecular pathology and nature of substrate accumulation.

The key function of lysosomal enzymes is the degradation of materials that is together with secondary lysosomes by fusion of the primary lysosome with phagosome or by other processes as shown in *figure 1.1.* LSDs are the consequence of an abnormal storage of undigested cellular debris, proteins, fats, carbohydrates and nucleic acids within the cell (Vellodi, 2005; Parkinson-Lawrence *et al.,* 2010). They occur due to mutations in the genes that encode for specific lysosomal hydrolases, resulting in an attenuated enzyme activity and/or their transport to the lysosomes (Vellodi, 2005; Saftig and Klumperman 2009). Currently, this group of combined pathology and resultant diseases are known as LSDs. This includes almost 60 different rare disorders, all of them genetic, and most of them are autosomal recessive except Fabry, Hunter and

Danon disease (Coutinho and Alves 2016).

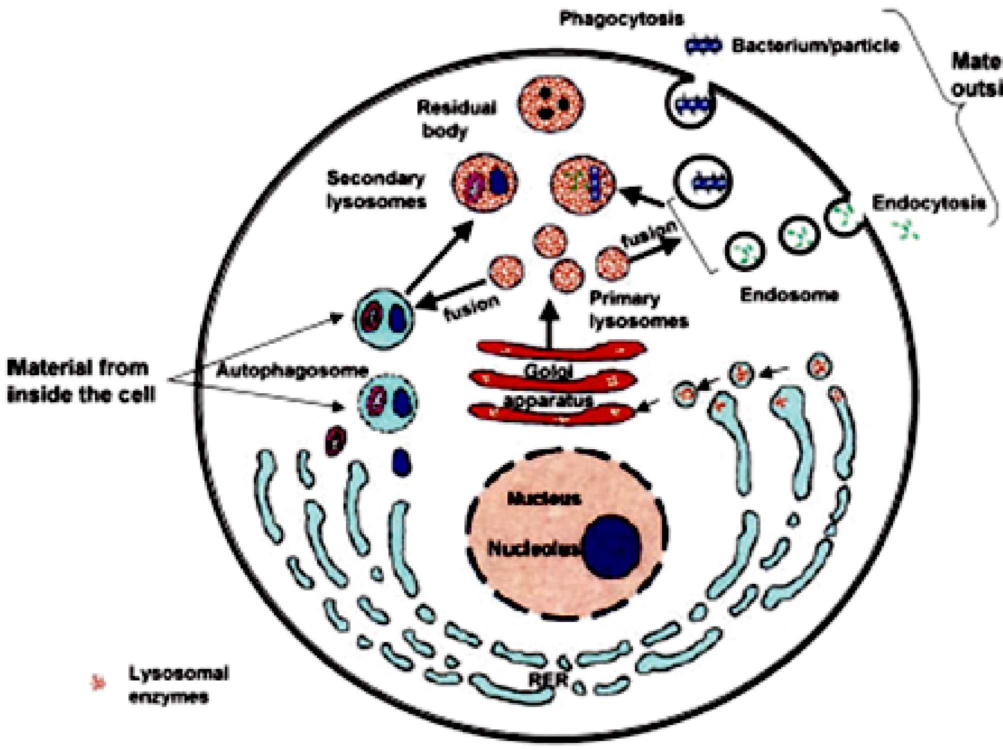

Figure 1.1: *The endocytic pathway (Vellodi, 2005)*

Individually, each LSDs are rare but collectively, they are as common as other metabolic disorders with a reported incidence of 1:5,000 to 1:7000 live births (Fuller *et al.*, 2006). The overall incidence of LSDs is 1:4,000 in northern Portuguese population; 1:7,100 in Netherlands; 1:7,700 in Australia; 1:8,100 in Czech Republic and 1:8,200 in Italy (Pinto *et al.*, 2004; Poorthuis *et al.*, 1999; Meikle *et al.*, 1999; Dionisi-Vici *et al.*, 2002; Poupětová *et al.*, 2010). The most frequent single LSD is Gaucher disease in Czech Republic, Italy and Australia, GM2 gangliosidosis in Portugal, Batten disease in Finland and Pompe disease in Netherlands. Newborn screening program in Taiwan identified a high frequency of Taiwanese males with Fabry disease (~1 in 1,250). It is likely to be due to high prevalence of cryptic mutation IVS4+919 G>A that was identified in 86% of patients with late-onset cardiac phenotype (Hwu *et al.*, 2009). Equally, Gaucher disease is also frequently observed genetic disorder in Ashkenazi Jews with an incidence of 1:855 live births (Staretz-Chacham *et al.*, 2009).

Few studies from India (Verma, 2000; Sheth *et al.*, 2004a; Nalini and Christopher, 2004; Verma *et al.*, 2012) have partly addressed the incidence of LSDs in India. Our group demonstrated that the burden is much higher (34.8%) in high risk children and in families which is similar to other European countries and Australia (Sheth *et al.*, 2014a). Among all LSDs, TSD (OMIM# 272800) was found to be the second most common lipid storage disorder amongst studied in India (Sheth *et al.*, 2014a). Though, this could be due to selection and referral bias in the study population. The present study was commenced to establish enzyme based diagnosis for patients affected with TSD and to identify disease causing mutations in *HEXA* gene. This will help to demonstrate genotype-phenotype correlation and establish a simple, cost effective method for the most commonly prevalent disease caused by mutations that can be utilized for carrier screening in mass population of Indian subjects.

1.1.2 Tay-Sachs disease: An introduction

Tay-Sachs disease (TSD) (OMIM# 272800) is an autosomal recessive neurodegenerative disorder caused by deficiency of β-hexosaminidase-A (Hex-A) owing to mutation in the *HEXA* gene (MIM* 606869). The gene encodes α-subunit of Hex-A, a lysosomal enzyme composed of α- and β- polypeptides (Tanaka *et al.,* 1999). The resulting phenotype of TSD can be variable with an acute infantile form of early onset leading to rapid neuroregression and early death to a progressive later onset form compatible with a longer survival. Clinical features include presence of neuroregression, generalized hypotonia, exaggerated startle response and cherry-red spot on the fundus. Affected patients have deficient enzyme activity of Hex-A in leukocytes/plasma/fibroblasts (Fernades and Shapiro, 2004). The human *HEXA* gene is located on chromosomes 15q23-q24 encompassing 14 exons. As per HGMD (Human Gene Mutation Database), nearly 170 mutations have been reported so far in the gene that causes TSD (http://www.hgmd.cf.ac.uk/). Majority of these mutations are private and are present in a single or a few families. Only a few mutations have been frequently reported for TSD in certain ethnic communities or geographically isolated populations. In Ashkenazi Jews, one of the three common mutations i.e. c.1278insTATC (p.Y427IfsX5), c.1421+1 G>C and c.805 G>A (p.G269S) have been reported in 94 - 98% affected patients (Myerowitz and Costigan, 1988; Kaback *et al.*, 1993).

The c.1278insTATC (p.Y427IfsX5) and the c.805 G>A (p.G269S) mutations are also commonly found in non-Ashkenazi Jewish populations, along with intron 9 splice site mutation c.1073+1 G>A, 7.6 kb French Canadian deletion (Myerowitz and Hogikyan 1986) and c.571 –1G>T in Japanese patients (Tanaka *et al.,* 1999). About 35% non-Jewish individuals also carry one of the two pseudo-deficiency alleles: c.739C>T (p.R247W) and c.745C>T (p.R249W), which is associated with absence of clinical phenotypes (Cao *et al.*, 1993) and deficient enzyme activity.

Molecular pathology of TSD in India has been partly addressed by our group as a part of present work (Mistri *et al.,* 2012; Sheth *et al.,* 2014a; Sheth *et al.*, 2014b). Our study was planned to identify novel and/or known mutations that will provide an insight into the molecular mechanisms of the *HEXA* gene in Indian subjects which in turn will facilitate carrier detection in the population and development of simple molecular technique for confirmative molecular diagnosis.

1.2 BIOLOGICAL RELEVANCE

1.2.1. Overview

1.2.1.1 History of Tay-Sachs disease (TSD)

TSD was described by a British ophthalmologist Warren Tay in 1881 and a physician Bernard Sachs, at New York’s Mount Sinai Hospital, in 1896 hence combined terminology as Tay-Sachs.

In 1981, a British ophthalmologist Warren Tay had noticed, a one-year old girl with mental and physical retardation. She had suffered from progressive muscle weakness since the age of three weeks and had deficient cerebral development. Ophthalmic examination saw cherry red spot in both retinas (Tay, 1881). The child was more critical and she died at the age of 18 months. An American neurologist, Bernard Sachs described as fatal disease commonly prevalent to Jewish patients that involved retardation and blindness, which he termed ‘amaurotic family idiocy’. He also noted some cellular changes like neurons from such patients seemed to have distended cytoplasm and ballooning of their dendrites (Sachs, 1887; Sachs, 1896).

Although, much evolution had been made in the understanding of TSD since then, scant information was available until discovery made by Klenk in 1942; a group of neuraminic acid containing sphingolipids that he termed gangliosides due to their high concentration in neurons or "ganglion" cells. He also noted that the TSD patients had accumulation of gangliosisde in brain tissue. The GM2 ganglioside accumulating is TSD was first identified in 1962, and the structure was further characterized three years later in 1965 by Ledeen and Salsman.

The clinical picture ranges from the acute infantile form to progressive later onset form compatible with a longer survival. Clinical features include neuroregression, generalized hypotonia and exaggerated startle response, seizures and cherry-red spot on fundus examination (Gravel *et al.,* 1995; Tropak and Mahuran, 2010).

Table 1.1: *Historical landmark in GM2 gangliosidosis*

Year	**Event**
1881	Warren Tay reports the first clinical description of condition (Tay, 1881)
1896	Bernard Sachs describes the “amaurotic family idiocy” prevent in the Jews (Sachs, 1896)
1942	Identification of a gangliosides stored in amaurotic idiocy (Klenk, 1942)
1962	GM2 ganglioside identified as the compound stored in TSD variant (Svennerholm, 1962)
1968	First report of late onset form, juvenile GM2 gangliosidosis reported in (Bernheimer and Seitelberger, 1968)
1969	Hex-A, but not Hex-B is deficient in TSD variants (Okada and O’Brein, 1969)
1969	Isoenzyme patterns in non-Ashkenazi Jewish patients: (a) deficiency of both Hex-A and -B (ββ); (b) no Hex deficiency (AB-variant or B1-variant) (Sandhoff, 1969)
1971	First prenatal diagnosis of Hex-A deficiency reported (Navon and Padeh, 1971)
1973	Immunological basis for the subunit composition of Hex-A (αβ) and -B (ββ): deficiency of the α-subunit results in TSD, and deficiency of β-subunit results in SD (Srivastava and Beutler, 1973)
1973-1974	Screening for carriers by automated Hex assay in serum (Lowden *et al.,* 1973; Kaback *et al.,* 1974)
1974	First clinical description of adult/chronic forms of the condition (Tallman *et al.,* 1974)

1978	Purification and characterization of the GM2 Activator protein: its deficiency results in the AB-variant form (Conzelmann and Sandhoff, 1978)
1984-1985	Isolation of cDNA clones encoding the α- and β-subunits (O'Dowd *et al.*, 1985)
1986	Identification of partial *HEXA* and *HEXB* gene deletions resulting in infantile TSD and SD variants (Myerowitz and Hogikyan, 1986; O'Dowd *et al.*, 1986)
1988	Identification of two major *HEXA* mutations responsible for TSD variant in Ashkenazi Jews (Arpaia *et al.*, 1988; Myerowitz and Costigan, 1988)
1989-1992	Isolation of a cDNA clone encoding the GM2 Activator (Schroder *et al.*, 1989; Xie *et al.*, 1991; Nagarajan *et al.*, 1992)
1991-1992	Association of GM2A mutation with AB-variants form (Schroder *et al.*, 1991; Xie *et al.*, 1992)
1995	Allogeneic bone marrow transplant in infantile form of GM2 gangliosidosis (Hoogerbrugge *et al.*, 1995)
1997-1999	Substrate reduction therapy showed to decrease the amount of GM2 ganglioside in brain of mice models (Platt *et al.*, 1997; Jeyukumar *et al.*, 1999)
2002-2006	First description of gene therapy in cell system and mice models (Martino *et al.*, 2002; Martino *et al.*, 2005; Cachon-Gonzalez *et al.*, 2006)
2009-2011	Primary research initiatives include gene therapy, development of ganglioside inhibitors, chaperone therapy, cord blood transplant, and enzyme replacement therapy (Rountree *et al.*, 2009; Clarke *et al.*, 2011; Matsuoka *et al.*, 2010)
2011	Lyso-GM2: Expected to be a potential biomarker of TSD and SD (Kodama *et al.*, 2011)
2012	The first gene therapy treatment was approved in Europe in November 2012 (Sandhoff, 2012)
2014	Chaperon therapy pilot study suggests cyclic low dose of Pyrimethamine (PMT) can increase Hex-A activity in late onset TSD patients. However, the observed increase is repeatedly transient and not associated with discernible beneficial neurological or psychiatric effects (Boyd *et al.*, 2013).

1.2.1.2 Gangliosides structures and locations

The typical chemical structure of a ganglioside is of ceramide attached to glycan chain via glycosidic bond at 1-hydroxyl place (Kolter, 2012) (*Figure 1.2*) containing 1-4 molecules of sialic acid (N-acetylneuraminic acid, NANA) residues (Schnaar et al., 2014) and are highly heterogeneous, resulting in high structural and functional diversity. The human brain has 12 different types of gangliosides, four of which are GM1, GD1a, GD1b and GT1b make up over

90-95% of the total (Tettamanti *et al.*, 1973; Ando *et al.*, 1978). Other brain ganglioside includes 9-O-acetyl GD3 as well as the complex forms GM2, GT1a and GQ1b (Kracun *et al.*, 1991; Yu *et al.*, 2012). These all gangliosides has the same tetra-saccharides chain to which one (GM1), two (GD1a and GD1b), or three (GT1b) sialic acid residues while GM2 and GM3 gangliosides contain a tri-saccharide chain as shown in *figure 1.3* (Mikata and Taniguchi, 1985; Sandhoff *et al.*, 1989).

Gangliosides are present in the plasma membrane of animal cells to which they are attached by their hydrophobic ceramide moiety. The oligosaccharide chain extends in to the extracellular space. The highest concentration of gangliosides occurs in cerebral gray matter and the pattern of mammalian brain ganglioside shows regional and developmental differences. Region of nerve ending and dendrites are rich of ganglioside, and most of the brain ganglioside is present in neurons, glial cells and other cell type (Suzuki, 1965). Gangliosides are also present in all known types of animal cells and participate in structural and physiological action (Colombaioni and Garacia-Gil, 2004; Zhang and Kiechle, 2004).

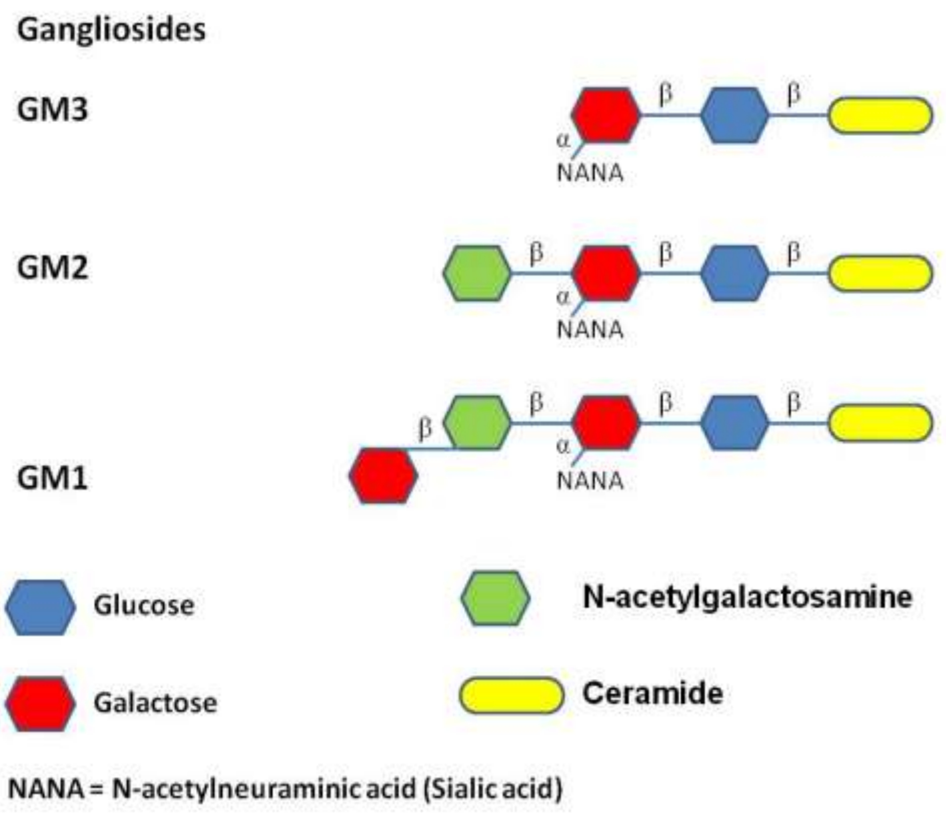

Figure 1.2: *Schematic ganglioside structure*

The gangliosidoses are a group of LSDs characterized by the accumulation of compound glycolipids in several organs of the body. Change in ganglioside expression are highly region specific (Wu *et al.,* 2005), strongly regulated and compared with neural tube formation, neurogenesis, synaptogenesis and myelination (Yu *et al.,* 2009; Yu *et al.,* 2012; Rahman, 1995), may probably be related to their high affinity for nervous tissues.

1.2.1.3 Biosynthesis and Catabolism of Gangliosides

In 1976, Fishman and Brady provided detailed explanation of ganglioside biosynthesis. It starts from ceramide which is formed at the cytosolic leaflet of the rough endoplasmic reticulum (ER) (Mandon *et al.,* 1992; Schnaar *et al.,* 2014). The glycosyl chain is assembled in a stepwise manner by sequential addition of the sugar and sialyl residues to the growing glycolipid (*Figure 1.3*). The glycosyl residues are donated by the respective uridine-5'-diphosphate (UDP) derivatives, while the active species for the sialyl residue is cytosine-5'-monophosphate (CMP-NeuAc) (Sandhoff *et al.,* 1989; Sandhoff, 2012).

Gangliosides are broken down to ceramides by sequential removal of sugar units in the oligosaccharide group which is catalyzed with a set of highly precise lysosomal enzymes. Mutations in functional gene impair the activity of enzymes leading to an accumulation of gangliosides in lysosomes leading to gangliosidosis disorder. A deficiency of any one of the lysosomal enzymes leads to stoppage of further breakdown; such as lipid storage diseases can result from a deficiency of any of the steps in the lipid degradation pathway. For example, the fatal TSD arises due genetic defect which leads to production of non-functional Hex-A, causing GM2 to accumulate in lysosomes and its accumulation in brain disturbs the normal functions of neurons (*Figure 1.4*) (Kolter and Sandhoff, 2010).

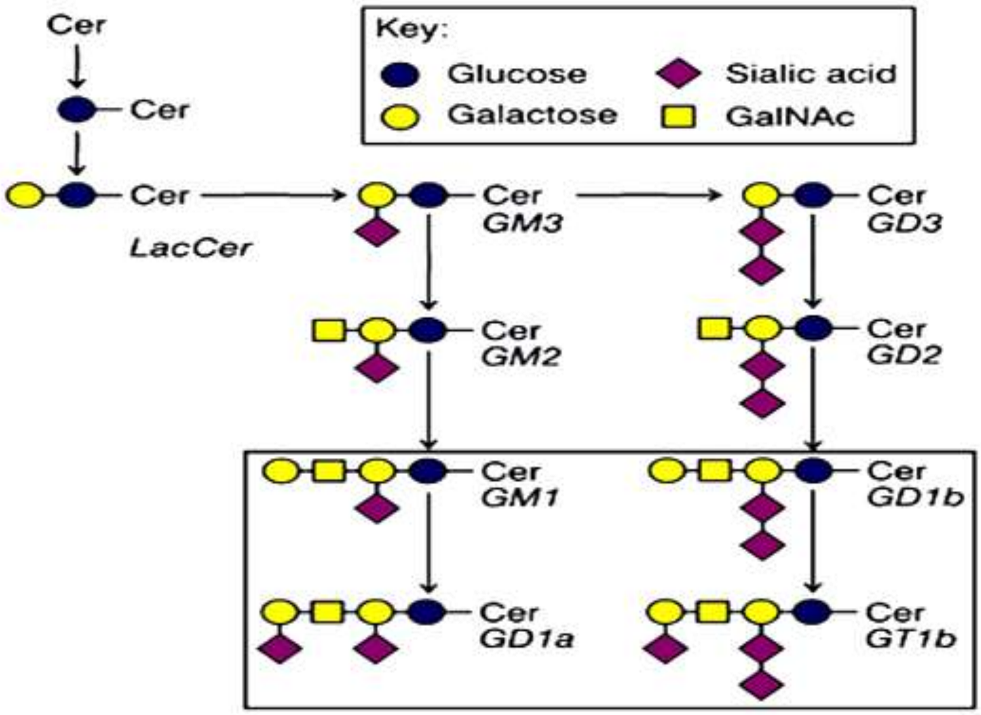

Figure 1.3: *Biosynthesis of brain Gangliosides.*

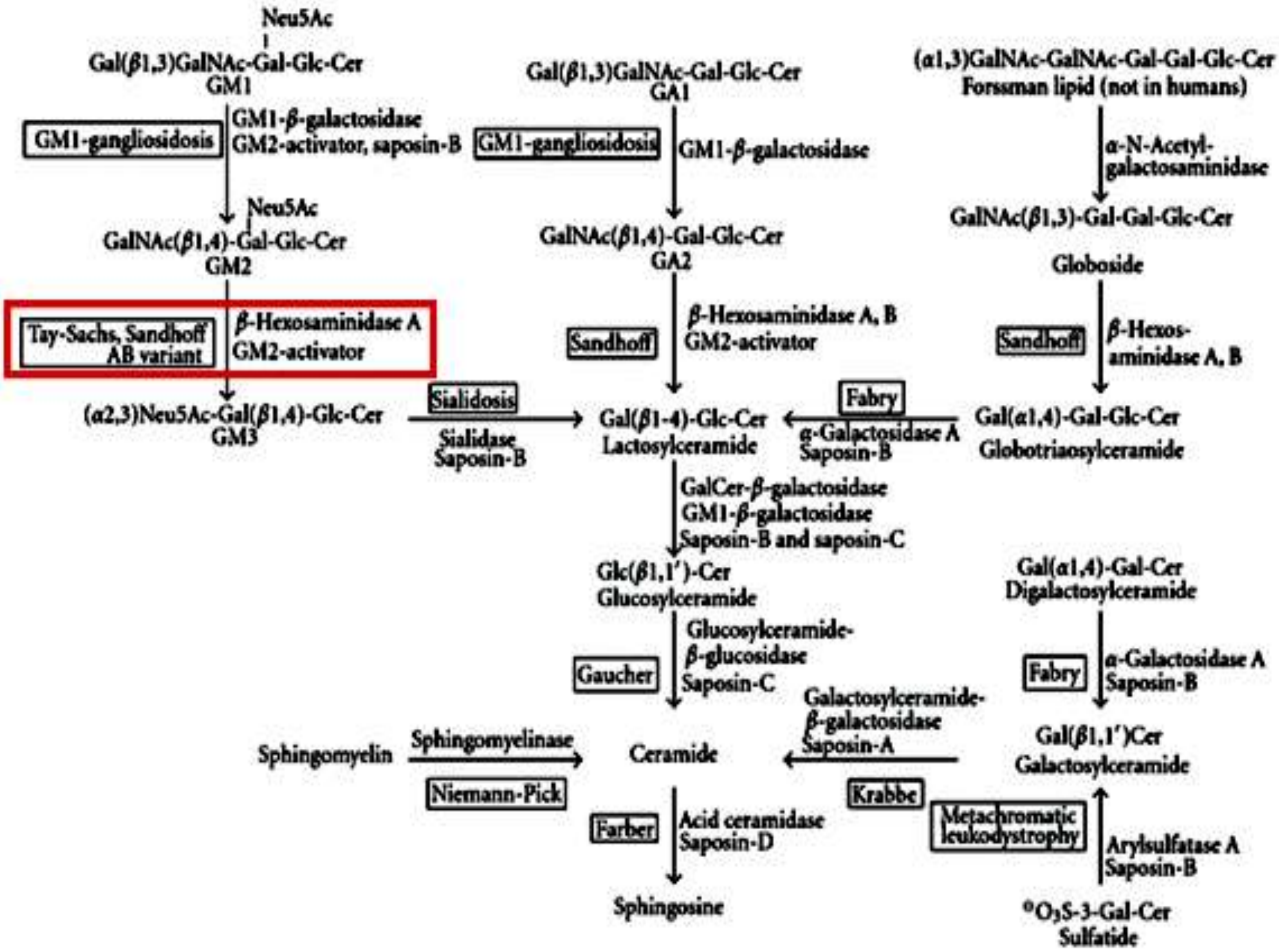

Figure 1.4*: Lysosomal ganglioside degradation pathway (Kolter and Sandhoff, 2006).*

1.2.1.4 Function of Ganglioside

The gangliosides are localized in outer leaflets of the plasma membranes in cells and are likely to be involved in cell differentiation and cell adhesion (Roseman, 1985; Varki *et al.,* 1991; Nelson *et al.,* 2005). The intracellular and intranuclear calcium homeostasis is also adjusted with the help of ganglioside (Ledeen *et al.,* 2008). Gangliosides are also present in most tissues and body fluids, also expressed in the nervous system (Yu *et al.,* 2009; Furukawa *et al.,* 2011). They are the significant glycolipids of neuronal plasma membranes and the surface pattern is created with the help of coordinated processes, biosynthetic pathways, catabolic steps of the endo-lysosomal system, and intracellular trafficking. As a result, the defects in ganglioside catabolism cause various fatal neurodegenerative disorders like GM1 and GM2-gangliosidoses (Sandhoff, 2012).

1.2.1.5 Gangliosides and diseases

Disturbed gangliosides are the pathology of many diseases. For example, Guillain-Barré syndrome is caused by an autoimmune response to cell surface gangliosides (Kaida *et al.,* 2009). In influenza (a well-known viral infectious disease), influenza virus A identify sialic acid residues of gangliosides which act as a receptor for invasion of host cells (Suzuki, 2005). LSDs, like GM1 gangliosidosis and GM2 gangliosidosis (Tay-Sachs disease, Sandhoff disease and GM2 activator protein deficiency) are caused due to deficiency of specific enzyme or co-activator protein, resulting in accumulation of the substrates like glycosphingolipids, including gangliosides in lysosome (Yu *et al.,* 2011). Moreover, Alzheimer's disease has also been shown to be initiated by aggregation of amyloid-β peptide caused by gangliosides. It is the most common neurodegenerative disease in adults (Matsuzaki *et al.,* 2010).

1.2.2 Biochemical consequence in Tay-Sachs disease (TSD)

1.2.2.1 Identification of the metabolic defect in TSD by the biochemical link between β-Hexosaminidase enzyme activity and GM2 gangliosidoses

The lysosomal degradation of gangliosides takes place by the set of specific glycosidase enzyme, which causes the sequential removal of non-reducing sugar at each newly produced terminal.

Therefore, since GM2 terminates in a β-linked N-acetyl galactosamine (*Figure 1.5*), it was hypothesized that TSD is attributable to the deficiency of β-hexosaminidase-A (Hex-A) enzyme. The artificial substrates have been identified to measure Hex activity with the most commonly used one today is 4-methylumbelliferyl-b-N-acetylglucosamine (MUG) and/or more specific sulphated substrate 4-methylumbelliferyl-N-acetyl-β-D-glucosamine-6-sulphate (MUGS) (Wendeler and Sandhoff, 2009). The fluorescent methyl umbelliferone (MU) is produced by the cleavage of β-GlcNAc from ganglioside structure, which makes a highly sensitive Hex assay (Wendeler and Sandhoff, 2009).

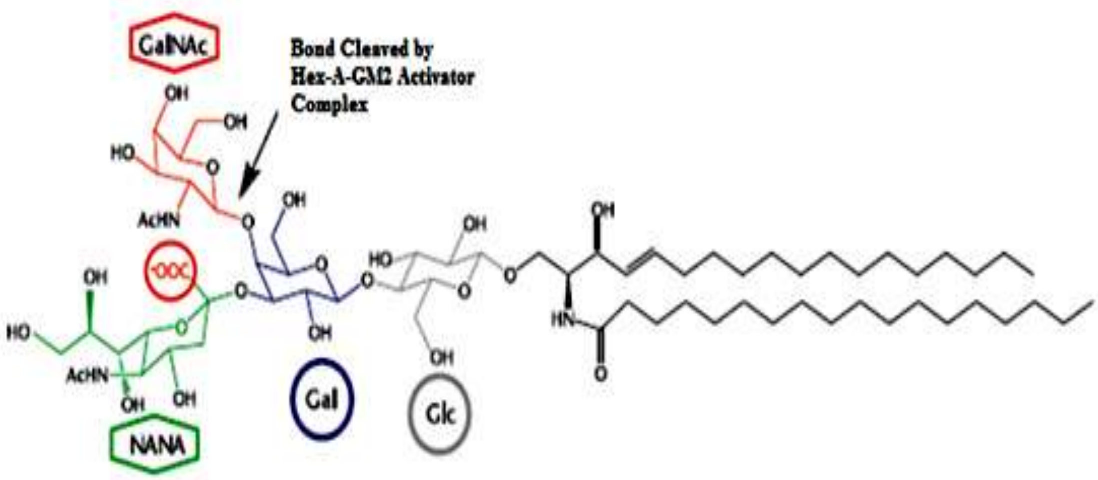

Figure 1.5: *Structure of GM2 ganglioside. [Tropak and Mahuran, 2010]*

In 1968, Robinson and Stirling demonstrated two hexosaminidase isozymes; heat labile Hex-A [with an acidic pI (4.8)] and heat stable Hex-B [with basic pI (6.9)]. Both had similar Michaelis-Menten constants (Km) and were present in the lysosomal fraction. In 1969, Okada and O'Brien found that Hex-A isozyme was deficient in Jewish TSD patients. However at the same time, Sandhoff had also observed that some samples had deficiency of Hex-A with normal Hex-B, few other had deficiency of both Hex-A and -B isozymes and still fewer had normal levels of both Hex-A and -B. However, the specificity of structure and substrate for each isozyme, and role of glycolipid transporter protein in GM2 hydrolysis and the GM2 activator protein were still under skepticism. They are elucidated as shown in *figure 1.6* (Mahuran, 1999).

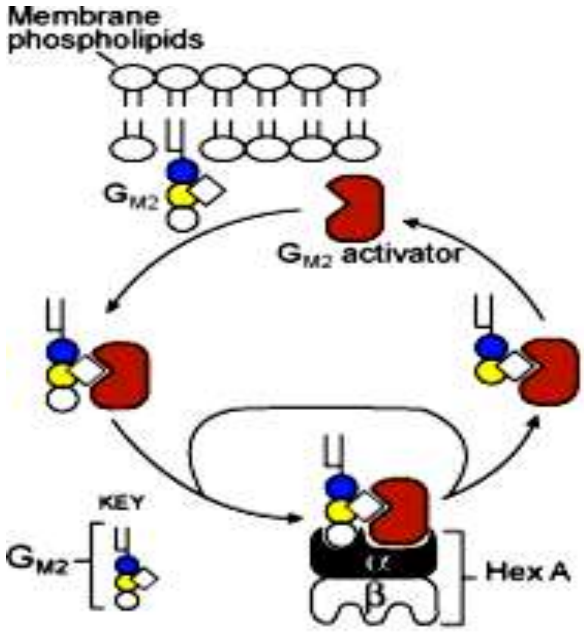

Figure 1.6: *Model for GM2 ganglioside metabolism. Under normal conditions, ß-hexosaminidase works in the lysosome of nerve cells to breakdown unwanted ganglioside GM2, a component of the nerve cell membrane. This requires three components: α-subunit, a ß-subunit and an activator subunit. In TSD, the α subunit of hexoasminidase malfunctions, leading to a toxic build-up of the GM2 ganglioside in the lysosome. [Chavany and Jendoubi, 1998].*

1.2.2.2 Subunit structure of Hexosaminidase

Initially, the structural relationship between Hex-A and Hex-B was suggested following observation that both isozymes are absent in SD. However, the exact nature of relationship was unclear. In 1968, Robinson and Stirling originally proposed that Hex-A and Hex-B were the same proteins; they differed only in their sialic acid content. Treatment of Hex-A with neuraminidase appeared to remove a NANA residue and convert this isozyme into the B form. This hypothesis was later disproved after it was shown that Merthiolate present in the neuraminidase preparations was converting the A to the B form; this conversion was accomplished even in the absence of neuraminidase (Carmody and Rattazzi, 1974). In 1973, Srivastava and Beutler also demonstrated that either in the presence or absence of neuraminidase, only 5 to 6 % of purified Hex-A was converted to a form resembling Hex-B in electrophoretic mobility. In 1974, another hypothesis proposed and suggested that the only difference between the Hex-A and -B isozymes was the degree of cross linking of the polypeptide chains by disulfide bridges (Tallman *et al.*, 1974).

The somatic cell hybridization studies confirmed the two-subunit theory between human and mouse cells. These studies demonstrated discordant segregation between Hex-A and Hex-B, signifying that the genes encoding the activities of the two isozymes were present on different chromosomes. Several scientists observed that expression of human Hex-B required only the presence of chromosome 5, since Hex-A required chromosome 5 and 15 simultaneously (Lalley *et al.,* 1974; Boedecker *et al.,* 1975; Chern *et al.,* 1976). This confirmed the two poly-peptide models and showed that the α- and β-subunits were encoded by distinct genes, *HEXA* gene, encoding the α-subunit, and *HEXB* gene, encoding the β-subunits (*Figure 1.7*).

An early immunological study by Srivastava and Beutler in 1973 concluded that both isoenzymes had a common subunit, Hex-A composed (αβ) of heteropolymer and Hex-B composed of (ββ) homopolymer. This examination was validated and developed by several scientists. It was finally concluded that loss of Hex-A and Hex-B activity in variant SD caused due to mutations in the β-subunit of gene (*HEXB* gene), whereas deficient activity of Hex-A in TSD is caused by mutations in α-subunit (*HEXA* gene) (*Figure 1.7*) (Sandhoff *et al.*, 2001). The minor and labile Hex-S has a specificity equal to that of Hex-A and necessary to hydrolyze anionic and neutral substrates, oligosaccharides and glycosphingolipids including the sulfated SM2 and ganglioside GM2 (Geiger *et al.*, 1977; Hepbildikler *et al.* 2002).

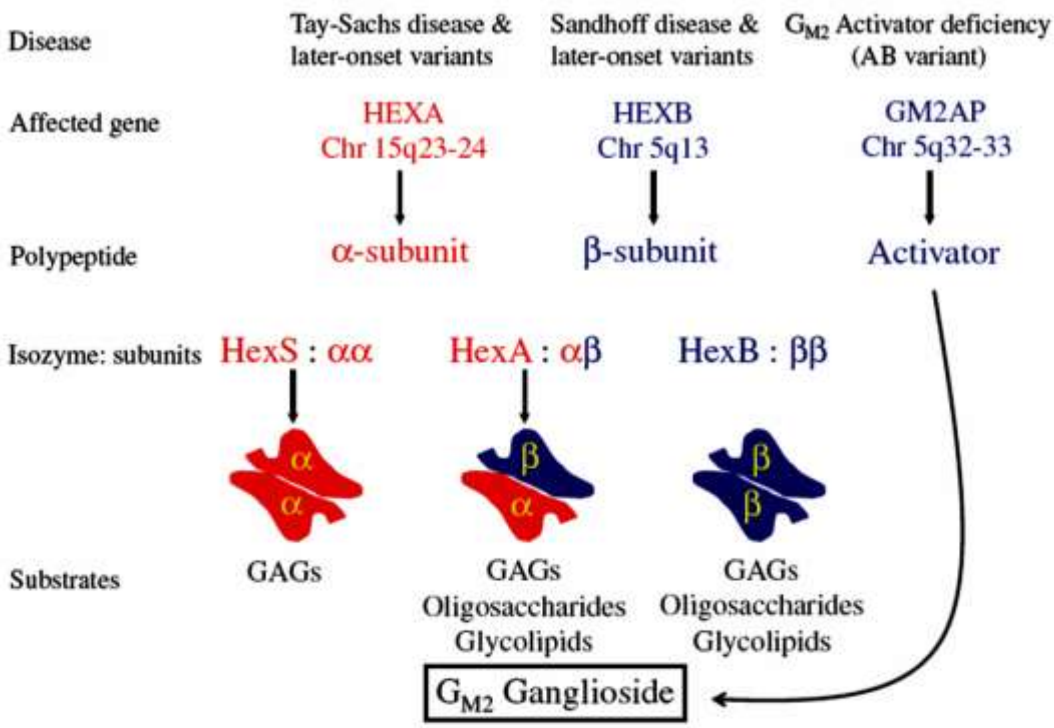

Figure 1.7: *The GM2 Gangliosidoses and the β-hexosaminidase system*
(Three polypeptides, each encoded in a different gene, are needed for the degradation of ganglioside GM2: the α- and β-subunit of the A isoenzyme and the activator protein which binds the ganglioside and presents it to the enzyme. (GAGs= glycosaminoglycans)[Sandhoff, 2012]

1.2.2.3 Isolation of cDNA clones for the subunits of Hex-A

In mid 1980s, the cDNA clones were isolated for the α- and β-subunits and the gene structure was determined a few years later. These data demonstrated that the *HEXA* (15q23-q24) and *HEXB* (5q13) genes evolved from a common ancestral gene resulting in the placement of all but the first of the intron/exon junctions lie in same positions in the genes and has a ~57% identity in a ligned, deduced amino acid sequences of the subunits (*Figure 1.8*) (Mahuran, 1999). The polymerase chain reaction (PCR) amplification methods of all exons and intron/exon junctions for mutational analyses of the *HEXA, HEXB* and *GM2A* genes have been reported earlier (Triggs-Raine *et al.*, 1991; Wakamatsu *et al.,* 1992; Chen *et al.,* 1999).

1.2.2.4 Substrate specificity of the Hex isozymes

In spite of their high degree of homology (*Figure 1.8*) only Hex-A can hydrolyse the non-reducing terminal β-linked GalNAc residue from GM2 ganglioside. In 1981, Kresse and his team noted that Hex-S and Hex-A, could hydrolyse the terminal β-linked GlcNAc-6-SO_4 from Keratan sulfate but Hex-B could not (Kresse *et al.*, 1981). These observations further helped to develop artificial substrate MUGS (MU-β-GlcNAc-6-SO_4) specific for Hex-A. However, MUG substrate is cleaved by all three isozymes and therefore is used to measure total-Hex activity. MUGS substrate is cleaved primarily by the α-subunit containing isozymes (Hex-A and -S). However, the β active site can slowly hydrolyze this substrate with MUG/MUGS ratios of 150–300: 1 for Hex-B, 3–4: 1 for Hex-A, and 1–1.5: 1 for Hex-S (Myerowitz and Hogikyan, 1986). Thus each of the %-residual Hex-A values is multiplied by ~3.6 (Hou *et al.,* 1996).

1.2.2.5 3D structure Hex-A/B

The first predicted 3D structure of the catalytic domain of human Hex was derived from bacterial chitobiase (Tews *et al.,* 1996). Determination of 3D structures of human Hex-A and Hex-B isozymes revealed that both α- and β-subunits have nearly similar structural properties. They are composed of two domain; domain-I of unknown function and domain-II, the catalytic core. Domain-I (αLeu23–αPro168, βAla50–βPro201) consists of a six-stranded anti parallel βsheet that overlays two parallel α-helices that built-up against two of the eight staves of the (β,α)8 (TIM Barrel) fold that forms domain-II (α165–α529, β202– β556) (*Figure 1.8*).

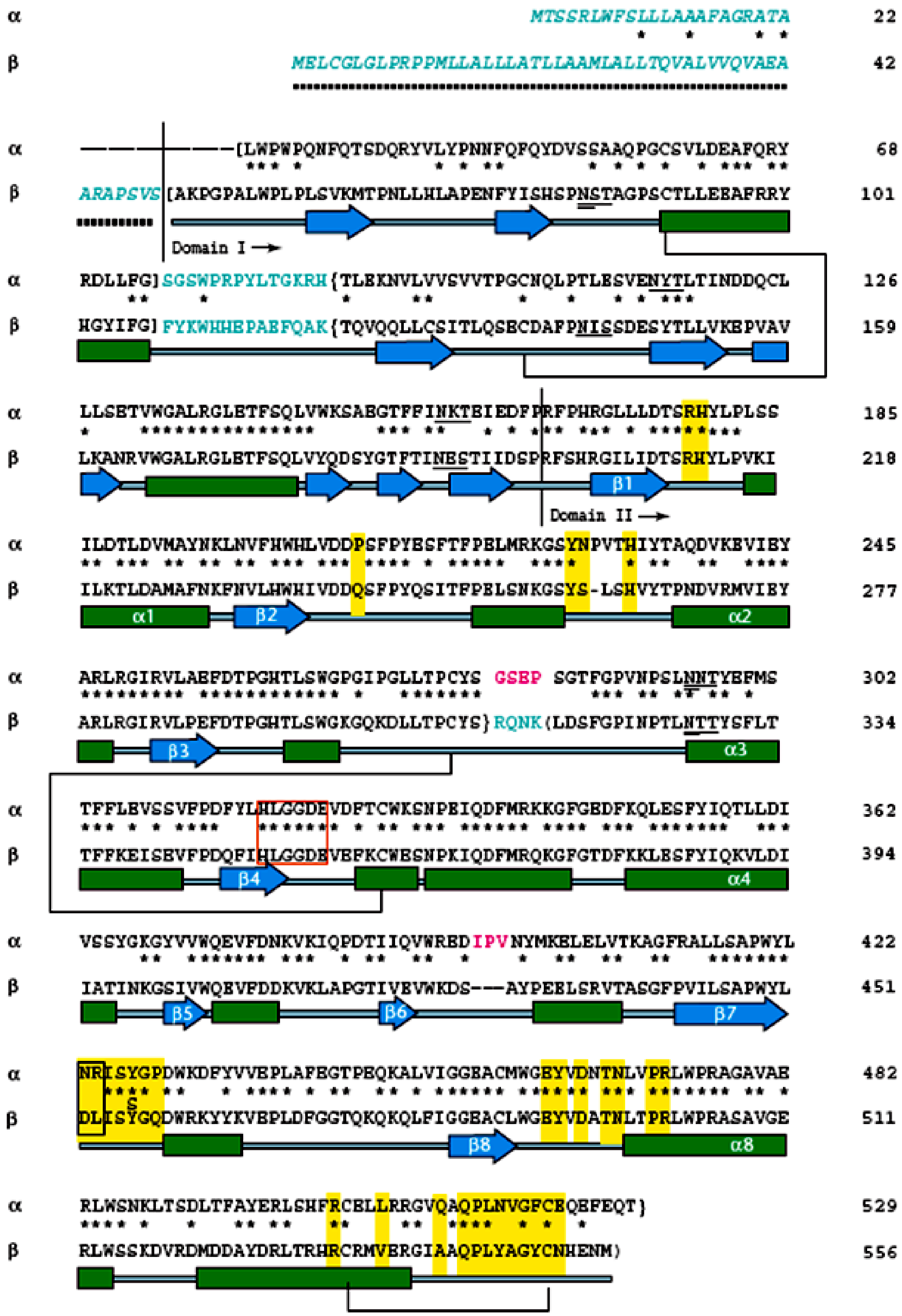

Figure 1.8: *Comparison and alignment of the amino acid sequences of the α- and β-subunits of human Hexosaminidase [Tropak and Mahuran, 2010].*

1.2.2.6 Biosynthesis and Lysosomal targeting of Hexosaminidase

A detailed explanation towards lysosomal enzyme targeting is provided by Kornfeld and von Figura and Hasilik in 1985 and 1986 respectively. Both α- and β-chains of Hex are synthesized as precursors in the endoplasmic reticulum (ER). Most of the enzyme binds to mannose-6-phosphate (M6P) receptors and is transported to the lysosome. In the lysosome, they are proteolytically processed into their mature forms, where the conformations of the proteolytic products are stabilized in part by the disulfide bonds (Hasilik and Neufeld, 1980). In SD, deficiency of β-subunit and normal levels of α-subunit were shown (O'Dowd *et al.,* 1985); however, only small increase was detected in monomeric α-precursor, no mature monomers were found, only low level of the mature homodimer (αα) could be detected or compared to normal cells (consist with 1-6% total-hex activity). This absence of α-subunit could not be explained by secretion (Proia *et al.,* 1984). These observations became understandable a few years later with the characterization of endoplasmic reticulum associated degradation pathway (ERAD) which is present in eukaryotic cells. This help in the regulation of protein quality control, by degrading unfolded and misfolded nascent protein, that are being targeted to other intracellular organelles (Rutishauser and Spiess, 2002). Hex subunits need to become correctly folded and form dimer in order to escape degradation by the ERDA. Therefore, the α-monomeric subunits in SD have a low affinity for each other and are likely to be degraded by ERAD. The different affinity of Hex subunits likely generates higher steady-state concentrations of properly folded α-monomers retained in the ER than β-monomer. It is likely to be preventing formation of the αβ heterodimers at a low concentration. Mutant protein of α- or β- subunits, found in either TSD or SD, may impair this steady-state subunit concentration in ER, and dramatically decrease the amount of mature Hex-A reaching the lysosome (Tropak and Mahuran, 2010).

It also implies that mutant proteins may not be totally incapable of forming a functional enzyme, but are prevented from doing so by being 'timed-out' by the ERAD system. Some α-subunit mutations like the αGly269Serin transfected COS cells have surprisingly high levels of enzyme activity, demonstrating that the ERAD system can be saturated (Brown and Mahuran, 1993; Mahuran, 1999). There are two isoenzyme of Hex-A, consisting of two subunits, α and β; and Hex-B, a homodimer of β-subunits. Only Hex-A can act on the GM2ganglioside/GM2 activator complex. Defects in any of three genes may lead to GM2 gangliosidosis: *HEXA*, which encodes the α-subunit of Hex-A; *HEXB*, which encodes the β-subunit of Hex-A and Hex-B; or *GM2A*, which encodes the GM2 activator. There are three forms: (i) TSD and variants caused due to mutations of the *HEXA* gene and associated with deficient activity of Hex-A with normal Hex-B; (ii) SD and variants caused due to mutation of the *HEXB* gene and associated with deficient activity of both Hex-A and Hex-B; and (iii) GM2 activator deficiency caused due to mutation of the *GM2A* gene and characterized by normal level of Hex-A and Hex-B but failure to form a functional GM2ganglioside/GM2 activator complex.

1.2.3 GM2 gangliosidosis

Various nomenclatures have been proposed to describe the different forms of GM2 gangliosidosis. In 1971, Sandhoff *et al.*, proposed a classification based on the hexosaminidase isoenzyme remaining in the tissue of affected patients: TSD (variant B) due to deficiency of Hex-A, SD (variant O) due to deficiency of both Hex-A and Hex-B, and variant AB due to GM2 activator defects. Currently, the three forms of the disease are classified as hexosaminidase α-subunit defects, hexosaminidase β-subunit defects and GM2 activator defects respectively (Sandhoff *et al.*, 1989). Mutations in the *HEXA* gene are associated with the most common TSD (carrier frequency of 1:30–40 Jews of Central and Eastern European extraction, and 1:300 for non-Jews), the *HEXB* gene mutations with the less common SD (carrier frequency estimated at 1:500 for Jews and 1:300 for non-Jews) and the *GM2A* gene mutations with a very rare AB variant form (Gravel *et al.*, 1995; Mahuran, 1999).

1.2.3.1 Tay-Sachs disease (TSD)

TSD is the manifestation of defective function in the α-subunit of *HEXA* gene that blocks the formation of intact Hex-A enzyme and the minor isoenzyme Hex-S (the αα dimer); however, the non-mutant β-subunits still associate to form Hex-B enzyme. The eponym TSD usually refers to the infantile form of the disease.

A few patients with GM2 gangliosidosis have been found to synthesize α-subunit that associates almost normally with β-subunits but is devoid of catalytic activity. The resulting dimmer, αβ, behaves like normal Hex-A in some respect such as isoelectric point and catalytic activity towards several synthetic substrate (MUG), but it is inactive towards the physiological MUGS substrate of GM2 gangliosides (Kytzia *et al.*, 1983; Li *et al.*, 1981). This variant, called as B1 variants was first thought to be related to GM2 activator deficiency, but was later shown to be allelic with other α-subunit deficiencies (Sonderfeld *et al.,* 1985).

Chronic GM2 gangliosidosis due to Hex-A deficiency was first described in 1968 (Bernheimer *et al.,* 1968). Since its original description, a number of cases have been reported of the chronic disease, in which the onset of symptoms of disease may be delayed form few years to decades.

1.2.3.2 Sandhoff disease (SD)

Patients with defects of the β-subunits have deficiencies of both isoenzyme activities (Sandhoff *et al.*, 1968; Sandhoff *et al.*, 1971). This disease has been also known as SD (O variants), indicating undetectable hex activity. This eponym SD is a reference to the first reported differentiation of this variant from classical TSD by Sandhoff and his colleagues (Sandhoff *et al.,* 1968). The small residual enzyme activity is due to the presence of α-subunit dimmers, called Hex-S (Beutler *et al.,* 1975) and is probably physiologically irrelevant.

The neurological features of SD and its variants are clinically indistinguishable from those occurring in TSD and variants. However, some infants with severe variants of SD also show clinical evidence of non-neurological symptoms like hepatosplenomegaly. The presence of occasional foamy histiocytes in bone marrow and occurrence of N-acetylglucoamine containing oligosaccharides in urine may also distinguish variants of SD (*HEXB* mutants) (Strecker *et al.*, 1977). Late-onset variants of Sandhoff disease have also been described in which the onset of symptoms is delayed for 2 to 10 years (subacute variants), or even into late adult life (chronic variants).

1.2.3.3 GM2 activator protein deficiency

GM2 activator deficiency is a rare disorder caused due to deficiency of the GM2 activator protein (Hechtman and Le Blanc, 1977). This is extremely rare condition with identical phenotype to infantile TSD. This tiny glycolipid transporter GM2 activator protein (GM2AP), acts as a substrate specific cofactor for the degradation of GM2 ganglioside by the enzyme β-hexosaminidase (*Figure 1.6*) (Mark *et al.,* 2003; Tropak and Mahuran, 2010). Till date, the infantile form of the disease has been identified; though it is very difficult to discriminate from infantile TSD or SD. A patient with apparently normal levels of Hex-A and -B, but with a TSD-like clinical phenotype may have GM2 activator protein deficiency (Conzelmann and Sandhoff, 1978). This disease is known as AB variant form of TSD.

1.2.4 Types and clinical description of Tay-Sachs disease

1.2.4.1 Infantile Tay-Sachs disease

It is caused by severe deficiencies in the catalytic activity of *HEXA* gene. It is the most common type of the GM2 gangliosidoses. The onset ensues in first few months of life, neuro-developmental delays followed by regression of milestones and degeneration of neurological deficits leading to early death (Sachs, 1887; Gravel *et al.*, 1995). Affected infants are normal at birth and have no clinical presentation up to 4 - 6 months. At four to six months, patients become less responsive to their environment and show decreased facial expression. The early sign is usually an increased startle response and seizures, which is later followed by generalized muscle weakness and hypotonia. The visual problem occurs at the age of one year. After two years of age, episodes of autonomic dysfunction with poor regulation of basic bodily function appear which tend to be a threatening sign of CNS involvement. Patients gradually develop spastic quadriplegia and a state of decelerate rigidity that leads to death within the first few years of life.

Neurological examinations reveals an encephalopathic-looking child with decreased responsiveness to environment stimuli, marked hypotonia, long tract signs in the form of hyperflexia, and a positive Babinski sign. Megalencephaly is also common, usually developing after first year of life. A cherry red spot with a white perimacular halo can be observed in both optic fundi by around the age of four months. This classical finding is due to accumulation of lipids around the macula, which leads to peripheral pallor and macular pseudo-prominence.

The most common neuroimaging findings are signal changes like hyperintensity or hypointensity in the basal ganglia and cerebral white matter on T2-weighted sequences on magnetic resonance imaging (MRI) studies (Brismer *et al.*, 1990; Fukumizu *et al.*, 1992; Yoshikawa *et al.*, 1992; Mistri *et al.*, 2012). Additional findings are also compatible with a process of demyelination, gliosis, and neuronal loss (Aydin *et al.*, 2005).

Availability of biochemical and genetic testing, skin biopsy, rectal biopsy for analysis of myenteric plexus neurons, or brain biopsies have been used for verification of the diagnosis (Takahashi *et al.*, 1987; Yamano *et al.*, 1982; Ul-Haque, 1995). Furthermore, membranous cytoplasmic body inclusions (MCBs) study on electron microscopy in neuronal cells and fibroblasts (Shirabe *et al.*, 1980). Moreover autopsy studies have shown severe cerebral and cerebellar atrophy, neuronal degeneration, and reactive gliosis. Presence of ballooned neurons and widespread neuronal loss were also observed in pathological examination (Moriwaki *et al.*, 1977). The megalencepahly was also observed at later stage (Johnson *et al.*, 1980).

1.2.4.2 Juvenile Tay-Sachs disease

The age at onset usually is between 3-5 years, mostly deterioration of daily activities, speech disturbances and gait disturbance (Brett *et al.*, 1973; Specola *et al.*, 1990). Regression of motor functions and cognitive abilities occur, leading to progressive neurological dysfunction and muscle weakness. Patients are usually identified with the disease by age 15 to 20 month of age, after a prolonged period in a persistent vegetative state. In some cases, the disease follows a particularly aggressive course culminating in death within 2 to 4 years of its onset. The cherry red spot is not consistently observed, and loss of vision occurs later in the acute infantile form. Optic atrophy and retinitis pigmentosa may also be seen later in the course of the disease.

Neuroimaging findings (MRI/CT) reveal minimally increased T2 signal in the white matter and a mild degree of cerebral and cerebellar atrophy also noted; however these findings are nonspecific for the disease.

1.2.4.3 Late-Onset or Adult Tay-Sachs disease

This type is typically presents in young adults. The clinical features are expressed at nearly 18 years with or without cognitive involvement (Neudorfer *et al.,* 2005; Frey *et al.,* 2005). Most common manifestation like cognitive involvement in the form of static or progressive dementia and gait disturbance were observed in nearly 44% patients (Frey *et al.*, 2005). These later features are associated to degenerative neurological defects. The well-known findings are lower motor neuron disease, weakness, proximal muscle wasting, cramps, and fasciculations (Harding *et al.*, 1987; Federico, 1987). Some other rare findings like psychiatric problem, late-onset spinal muscular atrophy, spinocerebellar disease, amyotrophic lateral sclerosis, or even Friedreich's ataxia are also reported (Turpin and Baumann, 2003; Noven *et al.,* 1977; Rubin *et al.,* 1988; Foster *et al.,* 1999; Perlman *et al.*, 2002; Drory *et al.,* 2003). Although these patients do not have cherry red spot but neuro-ophthalmologic manifestations like saccadic movements of eye are commonly observed (Rucker *et al.,* 2004).

Neuroimaging finding (MRI/CT) reveals cerebellar atrophy (Inglese *et al.,* 2005). Severe changes in brain phosphorus metabolism like decreased amount of phosphodiester and membrane bound phosphates have been also described (Felderhoff-Mueser *et al.,* 2001). The autonomic system abnormalities and sensory problems were also identified at later stage (Salman *et al.*, 2001; Barnes *et al.*, 1991).

The majority of adult onset patients carry a specific mutation G269S with some residual enzymatic activity to *HEXA*, and when present in homozygous state is associated with a milder clinical picture of later onset and slower progression (Neudorfer *et al.,* 2005; Paw *et al.,* 1989). Young patients presenting with this manifestation like slowly progressive speech problems, gait disturbance and unexplained psychiatric symptoms are highly likely to be affected by late onset or adult onset TSD.

1.2.4.4 B1 Variants of Tay-Sachs disease

Two forms; infantile and juvenile TSD have been reported with highly unusual presentation due to Hex-A deficiency (Riberio *et al.,* 1996; Maia *et al.,* 1990; Grosso *et al.*, 2003). In B1 variant, the Hex-A and -B activities are found to be normal with MUG substrate however deficiency of Hex-A is found toward MUGS substrate. The infantile and juvenile form of TSD, affecting the α-subunit (Hex-A activity) do not interfere with Hex-B activity on this basis they have been named as the "B variants" of the disease. The phenotype is very similar to classical TSD including the presence of megalencephaly and a macular cherry red spot in the early onset presentations. The degree of deterioration, however, seems to be faster once it appears, and death usually occurs within a few years. Neuroimaging findings are more remarkable in the infantile variants, revealing diffuse and symmetrical hyper-intensities of the cerebral and cerebellar white matter, posterior thalami, and corpus striatum (Grosso *et al.*, 2003). The corpus callosum looks thinner than normal, with residual and diffuse hypo-myelination leading to cortical atrophy at later stage. This is one of the common type of GM2 gangliosidosis, but has an exceedingly high incidence in the Portugal (dos Santos *et al.*, 1991), where specific mutation (p.R178H) has been reported.

1.2.5 The critical threshold hypothesis

Mutations result in to undetectable Hex-A activity, causes the acute form of GM2 gangliosidosis, while mutations allowing the production of even very small levels of residual Hex-A activity give rise to subacute or chronic diseases. Conzelmann and Sandhoff (1983) used a sensitive assay to demonstrate a correlation between the level of residual activity in cultured skin fibroblasts and clinical severity; infantile GM2 ≤0.1% of normal; severe juvenile, ≤0.5%; moderate-mild juvenile and adult GM2-gangliosidosis, 2-4%; healthy persons with "low Hex" 11% and 20% (Conzelmann and Sandhoff, 1983). These data suggests a 'critical threshold', i.e. the minimum amount of Hex-A activity required to keep the rate of GM2 hydrolyzed greater than or equal to the rate of ganglioside transport and incorporation into the lysosome, for TSD and SD of between 5% and 10% of normal Hex-A activity (Mahuran, 1999). Based on this principle, small but clinically significant increases in residual Hex-A levels may be helpful to a successful therapeutic approach.

1.2.6 Predominance of Tay-Sachs disease

1.2.6.1 International scenario

The TSD variant of GM2 gangliosidosis is the most common of the three variants of the disease with a prevalence of 1/201,000, and incidence of 1/222,000 live births (Meikle *et al.,* 1999). The SD prevalence and incidence were reported as 1/384,000 and 1/422,000 live births, respectively (Meikle *et al.,* 1999). It is found with a 10-fold higher frequency in Ashkenazi Jewish population (1:3600 versus 1:360000 in general non-Jewish populations) (Vellodi, 2005; Triggs-Raine *et al.,* 1990; Zimran *et al.,* 1991). The TSD carrier frequency is much higher in the Ashkenazi Jewish (1/30) and eastern Quebec French- Canadian populations (1/14), compared to the general population (~1/300) (Kaback, 2000). Community based TSD carrier screening programs in these at-risk populations has dramatically decreased the birth prevalence in these high-risk populations, which is now lower than that in the general population (Kaback, 2000; Mahuran, 1999). Recent carrier screening studies indicated that the frequency of the Ashkenazi Jewish founder mutations in individuals whose parents and four grandparents were Ashkenazi Jewish was 1:27.4, with the c.1278insTATC mutation accounting for about 80% of mutant alleles (Scott *et al.,* 2010). Moreover the incidence is approximately 1:320,000 newborns in the general population in United States (Tegay, 2012).

1.2.6.2 National scenario

The incidence or carrier frequency in India is not known for TSD but **a**mong all LSDs, it is the second most common lipid storage disorder studied in India (Sheth *et al.,* 2014a). However, mutation study was carried out for the first time by our group in India and found that three mutations c.1278insTATC (p.Y427IfsX5), c.1385A>T (p.E462V) and c.964G>T (p.D322Y) were commonly observed in ~42% patients (Mistri *et al.,* 2012; Sheth *et al.,* 2014a; Sheth *et al.,* 2014b).

1.2.7 Molecular Characterization of the *HEXA* Gene

Myerowitz and Proia (1984) isolated cDNA clone containing sequences complementary to Hex-A α-subunit mRNA. The clinical and biochemical identity of Ashkenazi and French Canadian Tay-Sachs phenotypes thus raised questions regarding the mutations in these two groups, and the possibility that TSD was introduced into the French Canadian population by the marriage of an Ashkenazi Jew. A cDNA clone containing the entire coding sequence of the α-subunit of Hex-A was subsequently isolated and characterized by Myerowitz *et al.,* in 1985. The cDNA is 1944 bp long, having a 5' untranslated region of 168 bp with an in-frame termination codon at nucleotides -85 to -87. A 529 amino acid open reading frame (Mr 60,697) corresponding to 1587 bp is initiated by a methionine-encoding triplet, and the amine terminus, rich in hydrophobic residues, is characteristic of signal sequences.

The *HEXA* gene spans approximately 35 kb of genomic DNA and is split into 14 exons. The exact locations and sequences of the intron/exon junctions have been determined. The α-chain gene has an expanded 5' end relative to its 3' end. Intron-1 is large, spanning more than 18 kb. The other introns range from 0.2 to 2. 1 kb. With the exception of exons-1 (>421 bp) and -14 (250/703 bp, resulting from the differential transcription and processing of this exons) that contain untranslated sequences, the exons show a restricted size distribution, ranging from 47 (exon-4) to 184 (exon-11) bp. Primers and the PCR conditions needed to amplify the complete *HEXA* gene exons and their flanking intronic sequences have been reported by Triggs-Raine and his colleagues in 1991.

1.2.8 Mutations in the *HEXA* gene associated with Tay-Sachs disease

The availability of a full-length cDNA probe permitted the analysis and comparison at the DNA level, of the α-chains in Ashkenazi Jewish and French Canadian patients. Southern blot analysis revealed a large 5’ deletion in the α-chain gene of French Canadians. The 5’ deletion was subsequently shown to be 7.6 kb long. It removes part of intron-1, all of exon-1 and extends 2 kb upstream, encompassing the putative promoter region. Although 70% of infantile TSD cases in French Canadians result from the 5’ deletion, more than one infantile TSD mutation occurs in this population (Hechtman *et al.,* 1990). A c.508 C>T (p.R170Q) mutation within exon-5 has also been identified in a French Canadian patient. In addition, c.805+1 G>A (IVS7 +1G>A) substitution abolishes the splice signal at the exon-7/intron-7 boundaries and has been identified in 13 French Canadian individuals in three families (Fernandes *et al.,* 1991). Two of the families trace their ancestry to the Charlevoix region of Eastern Quebec. The ancestry of the third family, which currently resides in Chicoutimi, has not yet been studied (Hechtman *et al.,* 1990).

The defect responsible for 70% of infantile TSD cases in Ashkenazim is an out-of-phase 4 bp insertion (c.1278insTATC) within exon-11, which generates an early termination codon, resulting in a deficiency of α-subunit mRNA (Myerowitz and Costigan, 1988). This mutation has also been detected in a French Canadian patient with no known Jewish ancestry (Hechtman *et al.,* 1990). A second, less frequent infantile TSD mutation in the Ashkenazi Jewish population is a c.1421 +1G>C abolishes the splice signal at the exon 12/intron 12 boundary (Arpaia *et al.,* 1988; Myerowitz and Costigan, 1988; Ohno and Suzuki, 1988a). These mutations have also been detected in Cajun families living in Louisiana (Mc Dowell *et al.,* 1989). The adult onset TSD in both Jewish and non-Jewish patients caused due to p.G269S mutation at the 3' end of exon-7 of *HEXA* gene. It is the third most common Ashkenazi TSD allele. Among Jewish patients, this allele is found in compound heterozygosity with one of the known infantile TSD alleles common in this population, whereas in non-Jews it occurs either in homozygous form or in combination with an unidentified mutation (Paw *et al.,* 1989; Navon and Proia, 1989; Navon *et al.,* 1990).

To date, nearly 170 different mutations of *HEXA* gene and 7 mutations of *GM2A* gene have been reported; and presented in public domain database shown in *table 1.2* and *table 1.3* respectively. Most of the mutations are private in nature, present in one or at the most in a few families of diverse backgrounds. Some are common in TSD carrier of particular ethnic origin or geographic location. Approximately 50% of the aberrations occur at CpG dinucleotide sequences corroborating their reputation as "hot Spot" for mutation via spontaneous deamination of methyl-cytosine to thymidine. The mutations present in the *HEXA* gene include 130 single base substitutions, 29 small deletions, 6 small insertions, 2 indels type mutations and 3 large deletion of 7.6 kb, Exon 2-3 deletion and Exon 1 deletion. The effect of these changes include substitution of amino acids (missense mutations), stop codon (nonsense mutations), abnormal splicing (splice site mutations), inclusion of incorrect sequences of amino acids (frame shifts mutations), deletions and insertions are listed below.

Table 1.2: Review of molecularly proven cases of Tay-Sachs disease

Sr. No	Nucleotide changes	Exon/ Intron	Amino acid changes	Clinical phenotype	Heritage	References
Missense Mutations						
1	c.1A>T	Exon-1	Met1Leu	Chronic (second allele=Gly269Ser	Japanese, Indian	Navon *et al.*, 1997 Present study
2.	c.1A>G	Exon-1	Met1Val	Acute	American Black	Mules *et al.*, 1992
3.	c.2T>C	Exon-1	Met1Thr	Subacute (second allele = Pro25Ser)	Non-Jewish	Harmon *et al.*, 1993
4.	c.10T>C	Exon-1	Ser4Pro	Likely Pseudo-deficiency	French Canadian	Wicklow *et al.*, 2004
5.	c.73C>T	Exon-1	Pro25Ser	Subacute (second allele = Met1The)	Non-Jewish	Harmon *et al.*, 1993
6.	c.109T>A	Exon-1	Tyr37Asn	Subacute (second allele = Met1Val)	African-American	Paciorkowski *et al.*, 2008
7.	c.116T>G	Exon-1	Leu39Arg	Acute	Non-Jewish	Akli *et al.*, 1993b
8	c.173G>A	Exon-1	Cys58Tyr	Acute	Iran	Najmabadi *et al.*, 2011
9.	c.320G>A	Exon-2	Glu114Lys	Acute	Indian	Mistri *et al.*, 2012
10.	c.379C>T	Exon-3	Leu127Phe	Acute	Various	Akerman *et al.*, 1997
11.	c.380T>G	Exon-3	Leu127Arg	Acute	Italian	Akli *et al.*, 1993b
12.	c.496C>G	Exon-5	Arg166Gly	Second allele = c.498delC	Syrian	Peleg *et al.*, 1995
13.	c.508C>T	Exon-5	Arg170Trp	Acute (second=Gly454Ser)	Italian, French-Canadian	Triggs-Raine *et al.*, 1991; Fernandes *et al.*, 1992; Akli *et al.*, 1993b
14.	c.509G>A	Exon-5	Arg170Gln + abnormal splicing	Acute	37% of TSD in Moroccan Jews; Japanese, Scottish	Akli *et al.*, 1993a; Nakano *et al.*, 1990; Drucker *et al.*, 1992

15.	c.524A>C	Exon-5	Asp175Ala	Acute	Indian	Sheth *et al.*, 2014b
16.	c.532C>T	Exon-5	Arg178Cys	Acute B1 variant phenotype	Czech, Indian	Tanaka *et al.*, 1990a Sheth *et al.*, 2014b
17.	c.533G>A	Exon-5	Arg178His	Acute B1 variant phenotype	Common in Portuguese, Diverse	Brown *et al.*, 1989; Ohno *et al.*, 1988; Tanaka *et al.*, 1990a;
18.	c.533G>T	Exon-5	Arg178Leu	Acute B1 variant phenotype	UK	Triggs-Raine *et al.*, 1991
19.	c.538T>C	Exon-5	Tyr180His	Chronic	Ashkenazi Jewish	De Gasperi *et al.*, 1996
20.	c.574G>C	Exon-6	Val192Leu	Acute second allele 9-IVS+1	European	Hou *et al.*, 1996; Ainsworth and Coulter-Mackie, 1992
21.	c.587G>A	Exon-6	Asn196Ser	Unknown with second allele Ser3Ser	French Canadian	Triggs-Raine *et al.*, 1995
22.	c.590A>C	Exon-6	Lys197Thr	Chronic (second allele = Arg499His)	Dutch	Akli *et al.*, 1993a
23.	c.598G>A	Exon-6	Val200Met	Acute	European	Gordon *et al.*, 1988
24.	c.607T>G	Exon-6	Trp203Gly	Acute with (second allele = F354del)	Italian	Montalvo *et al.*, 2005
25.	c.611G>A	Exon-6	His204Arg	Acute	German	Akli *et al.*, 1993a
26.	c.616G>C	Exon-6	Val206Leu	Acute	Indian	Present study (Unpublished data)
27.	c.621T>G	Exon-6	Asp207Glu	Acute	Non-Ashkenazi	Giraud *et al.*, 2010
28	c.623 A>T	Exon-6	Asp208Val	Acute	Argentinean	Zampieri *et al.*, 2012
29.	c.629T>C	Exon-6	Ser210Phe	Acute	Algerian, African	Akli *et al.*, 1991; Poenaru and Akli, 1994
30.	c.632T>C	Exon-6	Phe211Ser	Acute (second allele =Arg499Cys)	Italian	Akli *et al.*, 1993a
31.	c.637T>C	Exon-6	Tyr213His	Acute	Indian	Present study

						(Unpublished data)
32.	c.677C>T	Exon-6	Ser226Phe	-	Non-Jewish	Akerman *et al.*, 1997
33.	c.736G>A	Exon-7	Ala246Thr	Acute (second allele =Lys127Arg)	Italian	Montalvo *et al.*, 2005
34	c.739C>T	Exon-7	Arg247Trp	Asymptomatic in compounds with null allele	Diverse, ~5% of enzyme- based carriers in non-Jews, <3% in Jews	Cao *et al.*, 1997 ; Triggs-Raine *et al.*, 1992 ; Tomozak and Grebner, 1994; Kaback *et al.*, 1993; Mules et al., 1991; Triggs-Raine *et al.*, 1995
35	c.745C>T	Exon-7	Arg249Trp	Asymptomatic in compounds with null allele	French Canadian	Triggs-Raine *et al.*, 1991; Cao *et al.*, 1997 ; Cao *et al.*, 1993; Kaback *et al.*, 1993
36	c.746G>A	Exon-7	Arg249Gln	-	French Canadian	Cao *et al.*, 1997 ; Kaback *et al.*, 1993
37	c.748G>A	Exon-7	Gly250Ser	Pseudo-deficiency?	French Canadian	Triggs-Raine *et al.*, 1995
38	c.749G>A	Exon-7	Gly250Asp	Subacute	Lebanese	Trop *et al.*, 1992; Hechtman *et al.*, 1989
39	c.749G>T	Exon-7	Gly250Val	Acute	Iraqi-Jewish	Karpati *et al.*, 2000
40	c.755G>A	Exon-7	Arg252His	Chronic	Portuguese	Ribeiro *et al.*, 1996
41	c.755G>T	Exon-7	Arg252Leu	Acute with second allele = 5-IVS-1	Japanese	Tanaka *et al.*, 2003
42	c.772G>C	Exon-7	Asp258His	Severe subacute	Non-Jewish	Fernandes *et al.*, 1992 Tse *et al.*, 1996
43	c.775A>G	Exon-7	The259Ala	Acute	Non-Ashkenazi	Park *et al.*, 2010
44	c.788G>C	Exon-7	Thr263Ile	Acute	Indian	Sheth *et al.*, 2014a
45	c.805G>A	Exon-7	Gly269Ser	Adult onset	Ashkenazi 3%, 5% in non-Jews	Brown and Mahuran, 1993; Paw *et al.*, 1989;

					with TSD	Navon and Proia, 1989; D'Azzo *et al.*, 1984; Mules *et al.*, 1992; Navon *et al.*, 1990; Hund *et al.*, 1997
46.	c.805G>C	Exon-7	Gly269Arg	Acute (Second allele Asp175Ala)	Indian	Sheth *et al.*, 2014b
47.	c.806G>A	Exon-8	Gly269Asp	Adult onset	Non-Jewish	Akerman *et al.*, 1997
48.	c.835T>C	Exon-8	Ser279Pro	Severe subacute, second allele c.496delC	Israeli-Druze	Drucker *et al.*, 1997b
49.	c.885T>C	Exon-8	Asn295Ser	Acute with second allele = 5-IVS-1	Japanese	Tanaka *et al.*, 2003
50.	c.898T>C	Exon-8	Phe300Leu	NA	NA	Published In 2001 as per HGMD database
51.	c.902T>G	Exon-8	Met301Arg	Acute (second=Arg504His)	Yugoslavian	Akli *et al.*, 1993
52.	c.941A>T	Exon-8	Asp314Val	Acute	Ashkenazi Jews, Irish-English	Akerman *et al.*, 1997
53.	c.964G>T	Exon-9	Asp322Tyr	Acute	Indian	Mistri *et al.*, 2012 Sheth *et al.*, 2014a Sheth *et al.*, 2014b
54.	c.964G>A	Exon-9	Asp322Asn	Acute	Indian	Mistri *et al.*, 2012 Sheth *et al.*, 2014a
55.	c.965A>T	Exon-9	Asp322Val	Acute	Non-Jewish	Park *et al.*, 2010
56.	c.1003A>T	Exon-9	Ile335Phe	Affected prenatal	Non-Jewish	Tomozak and Grebner, 1994
57.	c.1121A>G	Exon-10	Gln374Arg	Acute	Italian	Montalvo *et al.*, 2005
58.	c.1121A>C	Exon-10	Gln374Pro	Acute	Indian	Sheth *et al.*, 2014a
59.	c.1164C>G	Exon-11	Ile388Met	Acute	French Canadian	Triggs-Raine *et al.*, 1995

60.	c.1171G>A	Exon-11	Val391Met	Chronic (second allele = Arg178His)	Greek	Navon *et al.*, 1995
61.	c.1178C>G	Exon-11	Arg393Pro	Subacute	Indian	Mistri *et al.*, 2012
62.	c.1260G>C	Exon-11	Trp420Cys	Acute	German	Tanaka *et al.*, 1990b
63.	c.1260G>T	Exon-11	Trp420Cys	Acute	Japanese	Tanaka *et al.*, 2003
64.	c.1351C>G	Exon-12	Leu451Val	Acute	Iraqi Jewish	Karpati *et al.*, 2004
65.	c.1360G>A	Exon-12	Gly454Ser	Acute (second allele = Arg170Gln	Italian	Akli *et al.*, 1993
66.	c.1360G>C	Exon-12	Gly454Arg	Acute	Indian	Present study (Unpublished data)
67.	c.1361G>A	Exon-12	Gly454Asp	Acute	Turkish	Ozkara *et al.*, 1998
68.	c.1363G>A	Exon-12	Gly455Arg	Acute	Portuguese	Ribeiro *et al.*, 1997
69.	c.1373G>A	Exon-12	Cys458Try	Acute	Japanese	Tanaka *et al.*, 1994
70.	c.1385A>T	Exon-12	Glu462Val	Acute	Indian	Mistri *et al.*, 2012
71.	c.1393G>A	Exon-12	Asp465Asn	Acute	Mexican	Alvarez-Rodríguez *et al.*, 2001
72.	c.1422G>C	Exon-13	Trp474Cys	Subacute (second allele=c.1278insTATC	Non-Jewish	Petroulakis *et al.*, 1998
73.	c.1432G>A	Exon-13	Gly478Arg	Acute (Second allele c.672 +30 T>G)	Indian	Mistri *et al.*, 2012
74.	c.1444G>A	Exon-13	Glu482Lys	Acute	2% of TSD in Moroccan Jews, Chinese, Italian	Akalin *et al.*, 1992; Kaufman *et al.*, 1997; Proia and Navon, 1992; Nakano *et al.*, 1988
75.	c.1451T>C	Exon-13	Leu484Pro	Acute	Japanese	Tanaka *et al.*, 1994
76.	c.1453T>C	Exon-13	Trp485Arg	Acute	Chinese	Akalin *et al.*, 1992
77.	c.1490A>G	Exon-13	Tyr497Cys	Acute	Saudi Arabia	Kaya *et al.*, 2011
78.	c.1495C>T	Exon-13	Arg499Cys	Acute (if second allele is Gly269Ser then chronic)	Slavic, Irish, English, Polish	Mules *et al.*, 1992; Akli *et al.*, 1993b

79.	c.1496G>A	Exon-13	Arg499His	Subacute	Scottish-Irish, Jewish	Triggs-Raine *et al.*, 1991; Akli *et al.*, 1993b; Paw *et al.*, 1990
80.	c.1510C>T	Exon-13	Arg504Cys	Acute	Diverse	Akli *et al.*, 1993a; Akli *et al.*, 1991; Tanaka *et al.*, 1994; Paw *et al.*, 1990
81.	c.1511G>A	Exon-13	Arg504His	Subacute	Diverse	Tanaka *et al.*, 1994; Paw *et al.*, 1990; Boustany *et al.*, 1991
82.	c.1511G>T	Exon-13	Arg504Leu	Acute	Irani	Zampieri *et al.*, 2012
Nonsense Mutations						
84.	c.78G>A	Exon-1	Trp26 Term	Acute	UK, Israeli, Arab	Drucker *et al.*, 1997; Triggs-Raine *et al.*, 1991
85	c.155C>A	Exon-1	Ser52Term	Acute	Irani	Zampieri *et al.*, 2012
86.	c.316C>T	Exon-2	Gln106Term	Acute	Various	Akerman *et al.*, 1997
87.	c.409C>T	Exon-3	Arg137Term	Acute	French, Irish	Mules *et al.*, 1992; Akli *et al.*, 1991
88.	c.540C>G	Exon-5	Tyr180Term	Acute	2% of TSD in Moroccan Jews	Drucker *et al.*, 1992
89.	c.709C>T	Exon-7	Gln237Term	Acute	Indian	Sheth *et al.*, 2014b
90.	c.987G>A	Exon-9	Trp329Term	Acute	English, German	Mules *et al.*, 1992
91.	c.1168C>T	Exon-11	Gln390Term	Acute	Ashkenazi Jews, Irish-English	Akerman *et al.*, 1997
92.	c.1176G>A	Exon-11	Trp392Term	Acute	Ashkenazi	Shore *et al.*, 1992
93.	c.1177C>T	Exon-11	Arg393 Term	Acute	French, Turkish	Akli *et al.*, 1991; Ozkara *et al.*, 1995
94.	c.1292G>A	Exon-11	Trp431Term	Acute with Second allele Met1The	Italian	Montalvo *et al.*, 2005

95.	c.1421G>A	Exon-13	Trp474Term	Acute	Indian	Sheth *et al.*, 2014a
96.	c.1426G>A	Exon-13	Arg476Term	Acute	Irani	Zampieri *et al.*, 2012
97.	c.1454G>A	Exon-14	Trp485Term	Acute	Indian	Sheth *et al.*, 2014a
98.	c.1528C>T	Exon-14	Arg510Term	Acute	Saudi Arabia, Iranian, Indian	Kaya *et al.*, 2011; Jamali *et al.*, 2014; Sheth *et al.*, 2014b
99.	c.1537C>T	Exon-14	Gln513Term	Acute	Irani	Zampieri *et al.*, 2012
Splicing Mutations						
100.	g→c	2-IVS+1	Abnormal splicing	Acute	European	Triggs-Raine *et al.*, 1991
101.	g→a	2-IVS+1	Abnormal splicing	Acute	French, Irish	Mules *et al.*, 1992; Akli *et al.*, 1991
102.	g→t	3-IVS+1	Abnormal splicing	Acute	Japanese	Tanaka *et al.*, 1994
103.	g→a	3-IVS-1	Abnormal splicing	Acute	Turkish	Ozkara and Sandhoff, 2003
104.	c→a	3-IVS-3	Abnormal splicing	Subacute	Mix European descent	Pierson *et al.*, 2013
105.	g→c	4-IVS+4	Abnormal splicing	Acute (Second allele Arg178Cys)	Indian	Sheth *et al.*, 2014b
106.	g→a	4-IVS+5	Abnormal splicing	Acute	French, Indian	Akli *et al.*, 1991; Sheth *et al.*, 2014b
107.	g→t	4-IVS-1	Abnormal splicing	Acute	US, Black	Mules *et al.*, 1991
108.	g→a	4-IVS-1	Abnormal splicing	Acute	India	Present study (Unpublished data)
109.	g→a	5-IVS-1	Abnormal splicing	Subacute	Tunisian	Akli *et al.*, 1990; Ozkara *et al.*, 1995

110.	g→a	5-IVS+1	Abnormal splicing	Acute	Turkish	Ozkara *et al.*, 1995;
111.	a→g	5-IVS-2	Abnormal splicing	Acute	12% of TSD in Moroccan Jews	Kaufman *et al.*, 1997
112.	g→t	5-IVS-1	Abnormal splicing	Acute	80% of TSD in Japanese	Tanaka et al., 1993
113	a→g	5-IVS-8	Abnormal splicing	Acute	Irani	Zampieri *et al.*, 2012
114.	g→a	6-IVS+1	Abnormal splicing	Sub acute	US	Akerman *et al.*, 1997
115.	t→g	6-IVS+30	Abnormal splicing	Sub acute	French Canadian, Indian	Triggs-Raine *et al.*, 1995; Mistri *et al.*, 2012
116.	g→c	7-IVS+1	Abnormal splicing	Acute	Portuguese, Indian	Ribeiro *et al.*, 1995; Mistri *et al.*, 2012
117.	g→a	7-IVS+1	Abnormal splicing	Acute (second allele = Δ7.6kb)	French-Canadian	Triggs-Raine *et al.*, 1991; Hechtman *et al.*, 1992
118.	t→c	7-IVS+2	Abnormal splicing	Acute	Italian	Montalvo *et al.*, 2005
119.	g→a	7-IVS-7	Abnormal splicing	Chronic (second allele = c.1278insTATC	Canadian/English	Fernandes *et al.*, 1997
120.	g→a	7-IVS+9	Abnormal splicing	Subacute	Israel	Levit *et al*., 2010
121.	t→a	8-IVS-15	Abnormal splicing	Subacute	French-Canadian	Wicklow *et al.*, 2004
122.	a→g	8-IVS+3	Abnormal splicing	Subacute (second allele = Arg178His)	Non-Ashkenazi	Richard *et al.*, 1995
123.	g→a	8-IVS+5	Abnormal splicing	Chronic	Non-Ashkenazi	Giraud *et al.*, 2010
124.	g→a	9-IVS+1	Abnormal	Acute	Major non-	Mc Dowell *et al.*, 1992;

			splicing		Jewish mutation; 8% of Cajun, 17% of W. Europeans	Landels *et al.*, 1992; Landels *et al.*, 1993; Akerman *et al.*, 1992; Akli *et al.*, 1993
125.	g→t	9-IVS-1	Abnormal splicing	Acute	Irish, French	Brown *et al.*, 1995
126.	a→g	10-IVS+18	Abnormal splicing	Acute	French Canadian	Triggs-Raine *et al.*, 1995
127.	c→t	11-IVS-26	Abnormal splicing	Subacute	Israel	Levit *et al.*, 2010
128.	g→a	11-IVS+1	Abnormal splicing	Acute	Italian	Montalvo *et al.*, 2005
129.	g→c	11-IVS+1	Abnormal splicing	Acute	French-Canadian	Boles *et al.*, 1995
130.	c→g	12-IVS+1	Abnormal splicing	Acute	15% of TSD in Ashkenazi, 2%in Morrocan Jews	Myerowitz and Costigan, 1988; Arpaia *et al.*, 1988; Kaufman *et al.*, 1997; Ohno and Suzuki, 1988a
Small deletions						
131.	411-Δagta	2IVS+4	Abnormal splicing	Acute (second allele c.910_912delTTC)	Non-Jewish	Giraud *et al.*, 2010
132.	c.423_424delTT	Exon-4	Δ142	Acute	French	Akli *et al.*, 1993
133.	c.426delT	Exon-4	Frame shift	Acute	Indian	Sheth *et al.*, 2014b
134.	c.436delG	Exon-4	Frame shift (exon-6-Stop)	Acute	US, Black	Mules *et al.*, 1992
135.	c.477_478delTG	Exon-5	Frame shift	Acute	UK	Triggs-Raine *et al.*, 1991
136.	c.496delC	Exon-5	Frame shift	Sub acute (second allele=Ser29Pro)	Israeli Druze	Drucker *et al.*, 1997

137.	c.498delC	Exon-5	Frame shift, Stop 96bp downstream	Second allele = Arg166Gly	Syrian	Peleg *et al.*, 1995
138.	c.613delC	Exon-6	Frame shift, Stop 6 bp downstream	Acute	Japanese	Tanaka *et al.*, 1999
139.	c.615delG	Exon-6	Premature stop codon	Acute with second allele= Arg504Cys	Italian	Montalvo *et al.*, 2005
140.	c.624_627TCCT	Exon-6	Frame shift	Acute	Irani	Zampieri *et al.*, 2012
141.	c.756_767del TGTGCTTGCAGA	Exon-7	Del 4 a.a.	Acute with second allele c.1278insTATC	Italian	Montalvo *et al.*, 2005
142.	c.910_912delTTC	Exon-8	ΔPhe304	Acute	43% of TSD in Moroccan-Jew (Irish, French)	Mules *et al.*, 1992; Akli *et al.*, 1991; Drucker *et al.*, 1992; Navon and Proia, 1991
143.	c.927_928delCT	Exon-8	Frame shift	Subacute	German, Scandinavian	Fernandes *et al.*, 1992
144.	c.958_961delGGA	Exon-8	ΔGly 320	Subacute	Irish	Mules *et al.*, 1992;
145.	c.898_905del TTCATGAG	Exon-8	Frame shift	Acute	Indian	Sheth *et al.*, 2014a
146.	c.1039_1056del GACTTCAAGCTGGAG	Exon-9	Deletion of 6 amino acids	Affected prenatal	Ashkenazi Jewish	Tomozak and Grebner,1994
147.	c.1043_1046delTCAA	Exon-9	Frame shift	Acute	Italian	Montalvo *et al.*, 2005
148.	c.1061_1063delTCT	Exon-9	ΔPhe354	Acute with second allele Gln203Gly	Italian	Montalvo *et al.*, 2005
149.	1074-Δtctcc	9-IVS-8-12	Abnormal splicing	Acute (second allele c.1278insTATC)	Polish	Triggs-Raine *et al.*, 1991;
150.	c.1096_1107del TATGGCAAGGGC	Exon-10	Delition of 4 amino acids	Acute	Turkish	Ozkara and Navon, 1998

151.	c.1123delG	Exon-10	Frame shift	Acute with second allele indels C168→A; ΔG170	Italian	Montalvo *et al.*, 2005
152.	c.1182delG	Exon-11	Frame shift	Acute	US	Tomozak and Grebner, 1994
153.	c.1204_1206delAAG	Exon-11	ΔLys402	Chronic		Akerman *et al.*, 1997
154.	c.1211_1212delTG	Exon-11	Frame shift	Acute	Non-Jewish	Giraud *et al.*, 2010
155.	c.1307_1308delTA	Exon-11	Frame shift	Acute	Irani	Zampieri *et al.*, 2012
156.	c.1334delC	Exon-12	Frame shift	Acute	Portuguese	Ribeiro *et al.*, 1995
157.	c.1470_1474delATCTG	Exon-13	Frame shift	Acute with second allele ΔPhe304	Italian	Montalvo *et al.*, 2005
158.	c.1510delC	Exon-13	Frame shift	Acute	Italian	Lau and Neufeld, 1989; Zokaeem *et al.*, 1987
159.	c.1613dupAAGT	13IVS+18	Frame shift	Subacute	European descent	Pierson et al., 2013
Small Insertions						
160.	+t	4-IVS+3t	Skips exon 4 early Stop, 17bp downstream	Acute	Israeli-Bedouin	Drucker *et al.*, 1997
161.	c.547insA	Exon-5	Frame shift	Acute	Chinese	Akalin *et al.*, 1992
162.	c.794insTC	Exon-7	Frame shift	Chronic	French-Canadian	Fernandes *et al.*, 1991
163.	c.947insA	Exon-8	Premature Stop	Acute	Italian	Montalvo *et al.*, 2005
164.	c.1278insTATC	Exon-11	Frame shift	Acute	20% of Non-Jews; 75% of Ashkenazi Jews; 92% of Cajuns; 2% of Moroccan Jews; 20% of Indian	Myerowitz and Costigan 1988; Mules *et al.*, 1992; Kaufman *et al.*, 1997; Mc Dowell *et al.*, 1992; Mistri *et al.*, 2012; Sheth *et al.*, 2014a; Sheth *et al.*, 2014b
165.	c.1549insC	Exon-14	Frame shift	Acute	Ecuadorian	Akerman *et al.*, 1997

Indels						
166.	C168→A; ΔG170	Exon-1	Pro170Pro + Frame Shift	Acute with Second allele ΔG1123	Italian	Montalvo *et al.*, 2005
167.	C496→G; ΔC498	Exon-5	Arg166Gly + Frame Shift	Chronic	Syrian	Peleg *et al.*, 1995
Large Deletion						
168.	Δ7.6kb	5’ to 1-IVS	Undetectable mRNA	Acute	82% of French-Canadian TSD	Myerowitz and Hogikyan, 1987; De Braekeleer et al., 1992; Hechtman *et al.*, 1990
169.	Exon-2 and 3 deletion	Exon-2 and 3	Undetectable mRNA	Acute	Indian	Present study (Unpublished)
170.	Exon-1 deletion	Exon-1	Undetectable mRNA `	Acute with second allele Glu462Val	Indian	Present Study (Unpublished)

* The mutations identified by Schroder et al. (1991) (CYS107ARG) and Xie et al. (1992) (CYS138ARG) are the same but derived from different amino acid numbering systems

Table 1.3: Review of molecularly proven cases of AB variant form of Tay-Sachs disease (GM2 activator protein deficiency)

Sr. No	**Nucleotide changes**	**Exon/ Intron**	**Amino acid changes**	**Clinical phenotype**	**Heritage**	**References**
			Missense Mutations			
1.	c.164C>T	Exon-2	Pro55Leu	Acute	Saudi Arabia	Salih *et al.*, 2015
2.	c.412T>C	Exon-3	Cys138Arg	Acute	US Black	Schroder *et al.*, 1991 Xie *et al.*, 1992
3.	c.506G>C	Exon-4	Arg169Pro	Acute	Indian	Schroder *et al.*, 1993
4.	c.522T>G	Exon-4	Leu174Arg	Acute	Indian	Kolodny *et al.*, 2008
			Nonsense Mutations			
5.	c.160G>T	Exon-2	Glu54Term	Acute	Laotian, Hmong	Chen *et al.*,1999 Renaud *et al.*, 2015
6.	c.472G>T	Exon-4	Glu158Term	Acute	Indian	Present study (Unpublished)
			Splicing Mutation			
7.	a→t	3-IVS-2	Abnormal Splicing	Acute	Indian	Present study (Unpublished)
			Splicing Mutations			
8.	c.262_264delAAG	Exon-3	ΔLys88	Acute	Saudi Arebia	Schepers *et al.*, 1996
9.	c.410delA	Exon-3	Frame Shift	Acute	Spanish	Schepers *et al.*, 1996

1.2.9 Genotype-Phenotype correlations

In TSD, majority of the genotypes are associated with acute infantile form of the disease, whereas few mutations are also associated with later onset, adult onset and B1 variants phenotypes. The first identified mutation 7.6 kb deletion was commonly originated in ~70% French-Canadian population (*Table 1.2*). The mutations common to the Jewish population were c.1278insTATC, c.1421+1 G>C and c.805 G>A (p.G269S) (Myerowitz and Costigan, 1988; Kaback *et al.*, 1993). The c.1278insTATC, c.1073+1 G>A and c.805 G>A (p.G269S) mutations were also observed in non-Ashkenazi Jewish and Cajuns populations (Myerowitz and Hogikyan, 1986). The mutations 7.6 kb deletion, c.1278insTATC, c.1421+1 G>C and c.1073+1 G>A are associated with the acute infantile form of TSD. The p.G269S mutation associated with adult TSD, accounts for ~3% of the mutant alleles in the Ashkenazi and ~5% in the non-Jewish population (*Table 1.2*) (Gravel *et al.*, 1995; Mahuran, 1999). Two missense mutations p.R247W and p.R249W are associated with pseudo-deficiency of Hex-A as shown in *Table 1.2* (Cao *et al.*, 1993). These mutations cause deficient Hex-A activity rather than lowering the specific activity of the protein, but not below the critical threshold needed to prevent GM2 accumulation (Mahuran, 1999). Moreover, Suzuki and colleagues identified the first specific mutation p.R178H associated with B1 variant form of TSD. Patients homozygous for this mutation present with a subacute phenotype, whereas those that are heterozygous with a second acute allele present with a noticeably more severe phenotype. Two other mutations at the same B1-codon have been described, p.R178C and p.R178L (Mahuran, 1999). In India, the most common mutations c.1278insTATC (p.Y427IfsX5), c.1385A>T (p.E462V) and c.964G>T (p.D322Y) are associated with infantile form of TSD (Mistri *et al.*, 2012; Sheth *et al.*, 2014a; Sheth *et al.*, 2014b).

The AB variants form of TSD (GM2 activator deficiency) is indistinguishable from infantile form of TSD due to its phenotypic similarity. Only nine mutations have been documented in the infantile form of *GM2A* gene till date and all patients were found to be homozygous for individual mutant alleles associated with complete absence of GM2 ganglioside cleavage (*refer table 1.3*)

1.2.10 Lacunae of Tay-Sachs disease

Therapeutic options for TSD represent a tremendous challenge to the medical community. Treatment options for TSD have rapidly expanded with limited success that include enzyme replacement therapy (ERT), substrate reduction, chaperone treatment, hematopoietic stem cell transplantation (HSCT), and gene-therapy (Tandon, 2002; Guidotti *et al.,* 1998; Shapiro *et al.,* 2009; Clarke *et al.,* 2011). Combination of treatments are also currently explored without clinically effective outcome likely attributable to CNS involvement. Since any such traditional mode of therapy requires access to the CNS (pharmacological chaperones) which can potentially stabilize the native folding of the protein despite its abnormal conformational changes, restore and protect the enzyme activity (Boyd *et al.,* 2013). For late infantile or adult onset TSD, pharmacological chaperones Pyrimethamine (PMT) was shown earlier to act as a Hex-A chaperone in human fibroblasts carrying some mutation like αG269S. The same treatment however was ineffective in subjects with late onset TSD associated mutations (Tropak and Mahuran, 2010).