CHAPTER - II
2. Review of Literature

Human Alpha Beta Hydrolase Domain (hABHD) family enzymes and their functions

Some of the human ABHD proteins were well characterized as lipases. A detailed explanation about their biochemical substrate specificity and physiological functional characterization are given below.

2.1. hABHD1 controls oxidative stress

hABHD1 is a protein of 45 kDa with 405 amino acids and is distributed across 9 exons. It is identified as a type II membrane protein (A.J. Edgar and J.M. Polak, 2002). No acyltransferase activity was found in ABHD1 and accordingly lapse the HXXXXD motif which is present in most of ABHD proteins. This protein was found to be highly expressed in heart and small intestine of mice. Though the enzyme biochemical functionality and substrate specificity has not been characterized. It has been found that over expression of hABHD1 in renal cell lines leads to the reduction of ROS by NADPH oxidative stress (M. Stoelting et al., 2009).

hABHD1 up-regulation is identified as a defensive reaction to regulate oxidative stress in oxidative stress induced hypertension mouse model (W.M. van Roon-Mom et al., 2008) where in hABHD1 expression is considerably up-regulated in kidney (J.C. Fowler et al., 2011). In addition hABHD1 is also found up regulated in developmental neurodegenerative disorder- huntington disease cell model (S. Kierstein et al., 2006). NRF2/ARE, an antioxidant pathway was found up-regulated in the above model controlling the oxidative stress as a protective mechanism (W.M. van Roon-Mom et al., 2008). Supportingly, mRNA levels of ABHD1 are also found upregulated in mice liver infected with parasites. ABHD1 was also found down regulated in small intestine and liver of transgenic mouse and was found involved in cell differentiation transcriptionally controlled by notch signalling. Hippocampal hABHD1 expression is down regulated by age and up regulated by exercise in mutant of Notch (ICD-E) transgenic mouse model (J.C. Fowler et al., 2011) hABHD1 was also down regulated in regenerative neurons in response to spinal cord injury in rats (R.A. Kohman et al., 2011). Collectively hABHD1 was found to have a significant role in maintenance of oxidative stress.
2.2. hABHD2 a key player in monocyte/ macrophage recruitment.

hABHD2 a type II membrane protein of 425 amino acids is found highly expressed in adrenal gland, brain and lungs of mice (A.J. Edgar and J.M. Polak, 2002). ABHD2 was initially found expressed in endothelial cells. The expression shifted to smooth muscle cells to suppress the cellular development of intimal hyperplasia. ABHD2 was down regulated in HIV patients treated with lamivudine, while the mRNA levels were found to be increased (S. Jin et al., 2009, K. Miyata et al., 2008, K. Miyata et al., 2005, X. Ding et al., 2011). In vivo function of abhd2 suppression was achieved by both gene trapping (S. Jin et al., 2009) and Anti-Sense Oligonucleotides (ASO) techniques (X. Ding et al., 2011, S. Jin et al., 2009). ABHD2 was also found to play a vital role in the replication of hepatitis B virus (X. Ding et al., 2011) and its propagation by knockdown of abhd2 in mice. Gene trapping—global deletion of human ABHD2 has led to the collection of macrophages in lungs and led to the decrease in the number of lung alveolar type II cells in aged mice. Deficiency of abhd2 led to an augmentation of inflammatory cytokines, reduced surfactant phospholipids, enhanced apoptotic cells and age related emphysema developed in mouse because of imbalance protease and anti protease ratio. It appears that role of the abhd2 is significant in macrophage penetration in to the lesions of atherosclerosis especially among CD68+ cells (K. Miyata et al., 2008). Though the function of ABHD2 is clear in atherosclerosis and emphysema (monocyte / macrophage) recruitment, the true physiological substrates of ABHD2 are not yet clearly understood and this enzyme is the major study target in the present thesis.

2.3. hABHD3 phospholipase

Human ABHD3 a 409 amino acid protein is located on chromosome 18q11.2 (A.J. Edgar and J.M. Polak, 2002). Similar to ABHD1, ABHD3 is also a type II membrane protein without HX4D motif. Human abhd3 is highly expressed in small intestine and brain of mice. ABHD3 showed activity with medium chain phospholipids. Phospholipase activity was proved with recombinant ABHD3 protein using C14 phosphatidylcholine in comparison with a serine ABHD3 mutant (S220A) which was catalytically inactive (J.Z. Long et al., 2011). Elevated levels of C14 phospholipids and phosphatidylcholine was observed in tissues of ABHD3 knockout mice (ABHD3 -/-) supporting the above activity. ABHD3 polymorphism let to the changes in phosphatidylcholine 32:2 concentration of human plasma which is in agreement as per the biochemical function identified (A. Demirkan et al., 2012). ABHD3 is upregulated in cancer cell lines of human ovaries in response to different chemotherapeutic targets and also
in breast cancer tumors (S. L'Esperance et al., 2008). Human osteosarcoma cell lines also shown ABHD3 over expression when HIC1 (Hypermethylated in cancer1) a tumor suppressor and also a transcriptional repressor is silenced (C. Van Rechem et al., 2009). In patients suffering from Crohn’s disease, ABHD3 is downregulated in peripheral blood mononuclear cells (N.Q. Fernandez-Becker et al., 2009). In a premature optic nerve wound of glaucoma rat model the expression of ABHD3 was down regulated (E.C. Johnson et al., 2011). Though physiological substrates of abhd3 are known its role in metabolic diseases remains unknown.

2.4. hABHD4 is a suppressor of cancer growth

Human abhd4, located on chromosome 14q11.2 is made up of 342 amino acids. This 39 Kda protein is universally expressed in mice multiple tissues including testis, brain, kidney and small intestine. C.A. Brady et al., (C.A. Brady et al., 2011) recently suggested that hABHD4 plays a key role in tumor suppression and cell proliferation. ABHD4 has shown lyso-N-acyl phosphatidyl ethanolamine lipase activity hydrolysing both NAPE and Lyso NAPE (C.A. Brady et al., 2011, G.M. Simon and B.F. Cravatt, 2006, J. Liu et al., 2008). AEA synthesis is induced by lipopolysaccharide (LPS) and there is no effect of ABHD4 knockdown on the LPS-induced AEA synthesis (J. Liu et al., 2008). Only 27 % increase of ABHD4 mRNA was found in cells treated with LPS for 90 minutes, signifying that ABHD4 leads only in the long term production of AEA but not in the production of endocannabinoid in response to LPS induction (J. Liu et al., 2008). ABHD4 was found upregulated in IVM oocytes supporting the role of abhd4 in suppression of tumor growth (M.G. Katz-Jaffè et al., 2009). Role of ABHD4 in cancer metastasis was confirmed when anchorage dependent cells were found dead upon disconnection of the extra cellular matrix evident by knockdown of abhd4 using shot hairpin RNA (RNAi).

2.5. hABHD5 guardian against Chanarin–Dorfman syndrome (CDS)

ABHD5 is 39kDa protein of 349 amino acid residues positioned on the chromosome 3p21.33, also named as CGI-58 (Comparative Gene Identification 58). hABHD5 is broadly expressed in mice, with maximum expression in testis and adiposites. NLSDI, a Neutral Lipid Storage Disease with Ichthyosis is a atypical non-lysosomal disorder. In NLSDI, excess triacylglycerol accumulation was shown, which is known as Chanarin–Dorfman syndrome caused by the mutation in ABHD5 gene (R.A. Igal et al., 1997, C. Lefevre et al., 2001).
Every recognized mutation in ABHD5 results in ichthyosis, hepatic steatosis, hepatomegaly and other neurological disabilities (M. Schweiger et al., 2009).

In hABHD5 nucleophilic serine was substituted by an asparagine. As a consequence, against triacylglycerols ABHD5 did not possess intrinsic hydrolase activity. The charge restrictive TAG hydrolase in mammalian adipose tissue, in the course of an unidentified mechanism was shown to particularly co-activate ATGL (Adipose Triacylglycerol Lipase) by ABHD5 (A. Lass et al., 2006). Additionally, it has been stated that ABHD5 is occupied in glycerophospholipid metabolism because of LPAAT function to produce the main indicated lipid phosphatidic acid (A.K. Ghosh et al., 2008, G. Montero-Moran et al., 2011). ABHD5 is restricted in the course of interface with perilipin-1 to the lipid droplet (V. Subramanian et al., 2004, T. Yamaguchi et al., 2004, T. Yamaguchi et al., 2007). Subsequently phosphorylation of perilipin-1 by cAMP-activated PKA through stimulation of lipolytic hormone (V. Subramanian et al., 2004, T. Yamaguchi et al., 2007), knockout mouse of human ABHD5 (ABHD5−/−) expired soon after delivery owing to a skin obstacle deficiency, which looks like ichthyosis in humans (F.P. Radner et al., 2010). Unpredictably, hABHD5 knockdown in adiposites consequences in lesser obese accumulation and totally stops diet-induced obesity (J.M. Brown et al., 2010), in dissimilarity to gentle obesity in ATGL−/−mice (G. Haemmerle et al., 2006).

The most important function of hABHD5 in adiposites might not be only the co-activation of triacylglycerol hydrolysis but is also mediated by ATGL. Fascinatingly, adipose tissue selective ABHD5 overexpression in mouse has shown negative results on weight of the body and did not enhance lipid hydrolysis (J.M. Caviglia et al., 2011). Knockdown of ABHD5 results in severe steatosis in the liver, yet inconsistently develops hepatic insulin signalling and systemic glucose tolerance (J.M. Brown et al., 2010, C.C. Lord et al., 2012). Provocative indicator pathways in the mice liver are considerably rounded by hABHD5 knockdown, while hepatic insulin signaling is enhanced (C.C. Lord et al., 2012). Significant changes in a number of glycerophospholipid species with signalling potential was revealed by lipidomic analysis of liver lipids, highlight the intention of hABHD5 functions to produce signaling lipids which can endorse confrontation of insulin (C.C. Lord et al., 2012). Even though a lot of development was seen in the function of hABHD5 perceptive, it is essential to clarify hABHD5 biochemical substrate specificity and physiological functions in metabolism of neutral lipids that might be different from co-activation of ATGL (C.C. Lord et al., 2012).
2.6. hABHD6 a regulator of endocannabinoid signaling

hABHD6 located on chromosome 3p21.1, is a 38 kDa protein prearranged by 10 exons, 2-arachidonoylglycerol hydrolase is the another name for hABHD6. In mouse, it is universally articulated with high expression in small intestine, brain and brown adiposites. hABHD6 is expected to have a single N-terminal transmembrane region and a cytosolic orientation. hABHD6 is the probable candidate gene for nuclear antigen 2 of Epstein–Barr virus (F. Li et al., 2009). In addition, expression of ABHD6 may be related to the Epstein–Barr virus pathogenesis associated disorders such as Hodgkin's, Post-Transplant and endemic Burkitt's lymphomas (F. Li et al., 2009, S. Maier et al., 2006). 2-arachidonoylglycerol (2-AG) is the primary substrate recognized for ABHD6 (J.L. Blankman et al., 2007, J.R. Savinainen et al., 2012, A. Straiker et al., 2011). 2-AG is an endocannabinoid signalling lipid that play a key responsibility in metabolic disease as well as in neurotransmission. 2-AG hydrolysis was long thought to be the role of monoacylglycerol lipase (MAGL), in recent times hABHD6 and hABHD12 both the enzymes have been shown to hydrolyze this important signaling lipid (J.L. Blankman et al., 2007, J.R. Savinainen et al., 2012, A. Straiker et al., 2011, W.R. Marrs et al., 2010, P. Zhong et al., 2011). hABHD6 regulates the motivated increase of 2-AG in whole neurons (J.L. Blankman et al., 2007, W.R. Marrs et al., 2010, P. Zhong et al., 2011, W.R. Marrs et al., 2011). In integral BV2 cells and BV2 cell homogenates, 2-AG hydrolysis reduced by ~50% through inhibition of ABHD6 (W.R. Marrs et al., 2010). Provided that ABHD6, ABHD12 and MAGL display diverse sub-cellular position, also assumed that every enzyme do somethings to arrange particular sub-cellular groups of 2-AG (J.L. Blankman et al., 2007, W.R. Marrs et al., 2010). Expression of ABHD6 was shown to be extremely related to Ewing family tumor linked gene (D. Max et al., 2009). Conversely, apoptosis rate, cell morphology and tumor growth velocity are unchanged with ABHD6 knockdown (D. Max et al., 2009).

2.7. hABHD7 a brain restricted epoxide hydrolase

hABHD7 is a protein of 42 kDa encoded by 7 exons located on chromosome 1p22.1, also known as epoxide hydrolase-related protein (EPHXRP) or epoxide hydrolase 4 (EPHX4). ABHD7 is identified as a type II membrane protein. An aspartate residue instead of serine is seen in hABHD7. Hence is expected to have activity towards epoxide containing lipid substrates as an epoxide hydrolase function. Epoxide holding lipids have strong bioactive results in progress of inflammation, blood pressure, pain and host defense (C. Morisseau,
2.8. **hABHD8 with unknown function**

hABHD8 is a 47 kDa protein located on chromosome 19p13.12. ABHD8 is vastly articulated in brain and testes of mice, mRNA of mice ABHD8 is pretty wealthy in all tissues inspected (Caleb C. Lord *et al.*, BBA, 2013). Biochemical substrate specificity and physiological function and subcellular localization of hABHD8 are presently unidentified. There is no experimental evidence to establish the biochemical substrate specificity and physiological function hABHD8.

2.9. **hABHD9 a predicted epoxide hydrolase**

hABHD9 is a 360 amino acid residue protein of 41 kDa. It is interpreted as an epoxide hydrolase similar to ABHD7, based on the aspartate presence instead of a serine. ABHD9 is universally present in mouse, with maximum expression in white adiposites, heart as well as skeletal muscle. A recent report finds out little levels of ABHD9 MRAA in skeletal muscle (M. Decker *et al.*, 2012). This difference might be due to detection of varying expression in different skeletal muscle sites or different variants of ABHD9. May be ABHD9 negatively regulate angiogenesis based on the recently identified endogenous substrates of ABHD9 like epoxyeicosatrienoic acids, angiogenic bioactive lipids (M. Decker *et al.*, 2012). Fascinatingly, in several types of cancers it was identified that hABHD9 was epigenetically silenced. For example, gastric cancers (S. Yamashita *et al.*, 2006), colorectal carcinomas (B. Oster *et al.*, 2011) and melanomas (J. Furuta *et al.*, 2006). Expression of hAHBD9 was also dysregulated in few B-cell malignancies (F. Micci *et al.*, 2007) and hABHD9 hypermethylation have been linked with reappearance of prostate cancer (S. Cottrell *et al.*, 2007, G. Weiss *et al.*, 2009). Based on these studies, hABHD9 biochemical substrates might be attached to development of cancer and it is possible. Interestingly, mutations in ABHD9 are expected to cause human ichthyosis (U. Ala *et al.*, 2008). hABHD9 also possesses activity in the direction of leukotoxin to produce metabolites that intercede acute respiratory distress syndrome (ARDS) (M. Decker *et al.*, 2012)
2.10. hABHD10 an enzymatic degrader of mycophenolic acid

hABHD10 is on chromosome 3q13.2 and a 306 amino acid residue protein (34 kDa) encoded by 5 exons. ABHD10 is all over present in mice tissues with maximum appearance found in brown adiposites and testes. ABHD10 is expected to be localized to mitochondria, but this has never been confirmed experimentally. hABHD10 was recognized as serine hydrolase which interacts with depalmitoylating enzymes in a study to recognize the targets of HDFP (hexadecyl fluorophosphonate) (B.R. Martin et al., 2012). To the liver, acyl-glucuronides are toxic and as a result hABHD10 might play an important role as a detoxification enzyme in liver (T. Fukami and T. Yokoi, 2012). In recent times, protein profiling has recognized ABL303, a little molecule inhibitor which is very efficient and choosy towards hABHD10 inactivation in vivo and in vitro (A.M. Zuhl et al., 2012). Developments with genetically modified animal models as well as inhibitors are needed evaluate biochemical substrate specificity and physiological functions of hABHD10 in the coming future.

2.11. hABHD11 invoved in Williams-Beuren syndrome

hABHD11 is a 315 amino acid residue protein of 35 kDa, formerly named as WBSCR21 (Williams-Beuren syndrome chromosomal region 21 protein). From truncated mRNAs, shorter isoforms of 325 amino residues are predicted, however at the protein level this is yet to be confirmed. hABHD11 has been noticed in mammalian mitochondria (N. Lefort et al., 2009). BV-2 cells have enriched levels of hABHD11 (W.R. Marrs et al., 2010). hABHD11 is universally present in mice with maximum availability in skeletal muscle, brown adiposites and heart. In Williams Beuren syndrome (WBS) several genes were deleted and ABHD11 is one of them. WBS patients have diverse neurodevelopmental features, including premature aging of the skin, opthalmic defects, cardiovascular disease, dysmorphic facial features, mental retardation, and distinctive cognitive and behavioral disabilities (G.Merla et al., 2002). This phenotypic feature result specifically from ABHD11 deletion or not is unknown so far. Elevated fat diet nourishing reduces the expression of hABHD11 in mouse white adipose tissue, while treatment of rosiglitazone increases the expression of ABHD11 (W.J. Shen et al., 2007). Additionally, expression of hABHD11 in brown adiposites is drastically condensed in HSL (hormone sensitive lipase) knockout mouse (W.J. Shen et al., 2007). Even though the endogenous substrates are unidentified in portraying the physiological function newly recognized inhibitors could be useful for this purpose. ABHD11 is identified as a potential biomarker in human lung adenocarcinoma, since increased hABHD11 activity in
lung cancer led to the expansion of isolated metastases (T. Wiedl et al., 2011). hABHD11 substrate specificity and functional identification might guide to enhanced realizing of the WBS pathogenesis.

2.12. hABHD12 is protector against PHARC development

hABHD12 is a 45 kDa protein located on chromosome 20p11.21. Previously named as c20orf22, hABHD12A and 2-AG hydrolase. Based on N-linked glycosidase digestion studies hABHD12 is expected to be a protein of integral membrane facing the extracellular space or ER (J.L. Blankman et al., 2007, J.R. Savinainen et al., 2012). In mice tissue, ABHD12 is universally expressed with maximum appearance in brain, where ABHD12 showed ~9% of whole hydrolase activity along with ABHD6 and MAGL (monoacylglycerol lipase) towards endocannabinoid 2-AG, (J.L. Blankman et al., 2007, J.R. Savinainen et al., 2012, W.R. Marrs et al., 2010). hABHD12 preferred 1 (3)- and 2- isomers of arachidonoylglycerol in the in vivo inhibitor profiling which also demonstrated that there is difference between hABHD12 and hABHD6 in substrate specificity (D. Navia-Paldanius et al., 2012). Particularly in microglia, hABHD12 transcripts are extremely articulated in different brain cell types (J.R. Savinainen et al., 2012). In osteoclasts and macrophages ABHD12 is abundant (J.R. Savinainen et al., 2012), and mRNA of hABHD12 is extremely rich and universally expressed in mice tissues inspected. Significantly similar to hABHD6, hABHD12 has no effect on the revival of DSE, however it emerges to interchange all through the neurons (A. Straiker et al., 2011). Fascinatingly, it has been shown that a neurodegenerative disease named PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract) in homosapiens was caused by mutations in hABHD12 (T. Fiskerstrand et al., 2010). Four diverse mutations in hABHD12 have been linked through PHARC enlargement signifying a fundamental relationship of genotype and phenotype (T. Fiskerstrand et al., 2010). It has been recommended that depending on its capability to hydrolyze the 2-AG enzymatically, hABHD12 defects leads to PHARC, as in neuroinflammation and synaptic plasticity (T. Fiskerstrand et al., 2010). Usher Syndrome 3 has been associated with a nonsense mutation in hABHD12 (T. Eisenberger et al., 2012). Ultimately, expression of hABHD12 has been linked with the development of colorectal cancer (T. Yoshida et al., 2010). Although, 2-AG successfully identified as one of the physiological substrate for ABHD12, biochemical function of this action remains undecided.
12.13. hABHD12B with unknown function

hABHD12B is a 362 amino acid protein of 41 kDa. Formerly known as C14orf29. From truncated mRNAs isoforms of hABHD12B lesser than 362 amino residues were predicted, the biochemical function and subcellular localization of ABHD12B has yet to be determined. Additionally, expression of ABHD12B has not yet been characterized in mice. Considerable downregulation of hABHD12B mRNA in skin cancers that causes mutations in the cancer suppressor gene CYLD is the solitary report on expression of hABHD12B in homosapiens so far (N. Rajan et al., 2011). Even though ABHD12B is predicted to cover both acyltransferase and hydrolase activities, physiological function and biochemical substrate specificities of hABHD12B are still unknown.

12.14. hABHD13 with unknown function

hABHD13 formerly known as C13orf6, is a 337 residue (39 kDa) protein encoded by 2 exons located on chromosome 13q33.3. hABHD13 might not have the acyltransferase function since its lacks a HX4D motif, but predictive of esterase activity because of the presence of a conserved catalytic triad. In the premature reaction to chemotherapy ovarian cancer cell lines hABHD13 is upregulated (S. L'Esperance et al., 2008). In addition, it has been reported that hABHD13 is controlled by circadian regularity in the liver mouse (R.R. Almon et al., 2008). At present, no data is available on the physiological function and biochemical substrate specificity of hABHD13.

2. 15. hABHD14A is involved in autism spectrum disorder

hABHD14A is a 271 amino acid protein of 30 kDa, previously known as Dorz1. It is predicted that ABHD14A could be a type II membrane protein. hABHD14A having preserved catalytic triad is expected to show esterase function although not anticipated to have only acyltransferase function since HX4D motif is missing. mRNA of hABHD14A is extremely expressed universally with maximum expression in testis when contrasted to previous tissues in mouse model. The physiological function and biochemical substrate specificity of hABHD14A are unidentified. Similar to hABHD11, hABHD14A might be implicated in the pathophysiology of WBS (Williams-Beuren syndrome). Expression of ABHD14A is elevated in patients of Williams-Beuren syndrome skin fibroblasts which are different compared to the removal of hABHD11 in Williams-Beuren syndrome (C.N. Henrichsen et al., 2011). Besides, hABHD14A is linked to autism spectrum disorder, which
is a genetical disorder (J.P. Casey et al., 2012). In mice, expression of hABHD14A associates powerfully in the cerebellum with neuronal development, signifying that hABHD14A might play an important function in the proliferation or segregation of neuron ancestors (J. Hoshino et al., 2007). It will be fascinating to establish the ABHD14A enzymatic function, and whether this is associated to the enlargement of either Williams-Beuren syndrome or autism spectrum disorder in human beings.

2.16. hABHD14B enzyme with large potential

hABHD14B is a 22 kDa protein, previously known as CIB (CCG1-interacting factor B). Truncated mRNAs isoforms lesser than 210 amino acids are forecasted, however this has yet to be established at the level of protein. Localization of ABHD14B to both nucleus and cytoplasm in transfected COS cells is shown by ABHD14B-GFP fusion proteins (B. Padmanabhan et al., 2004). In most mouse tissues, ABHD14B is lavishly and universally expressed, but maximum expression found in the kidney and liver only. In vitro hydrolase function in the direction of the familiar lipase substrate paranitrophenyl butyrate within a dosage dependent manner has been shown by hABHD14B (B. Padmanabhan et al., 2004). Though, true biochemical functionality unidentified in humans, the hABHD14B is hypothesized that it might acts as a transcription factor in eukaryotes (B. Padmanabhan et al., 2004, N. Posorski et al., 2011), however further work is required to validate its nuclear responsibility. It has been shown that expression of hABHD14B was high in metastastic neuroendocrine cancers (N. Posorski et al., 2011).

2.17. hABHD15 novel target Akt phosphorylation

hABHD15 is located on chromosome 17q11.2 and it is a 468 amino acid residue (52 kDa) protein encoded by 2 exons. hABHD15 shares the majority sequence relationship with human ABHDs hABHD1, hABHD2, and hABHD3. But, ABHD15 is not having an HX4D motif and a nucleophilic elbow. hABHD15 is in fact predicted to be a protein of unknown function. In recent times, hABHD15 has been portrayed as a probable original participant in insulin signalling (S. Gridley et al., 2005, J.A. Chavez et al., 2006). This discovery proposes that ABHD15 may turn out to be constant PDE3B to unenthusiastically control the levels of cyclic AMP. PDE3B is triggered by insulin signalling Akt phosphorylation (B. Omar et al., 2009). Under insulin stimulation conditions, cellular pool of cAMP reduces by PDE3B, abating hormone-stimulated lipolysis and protein kinase A activation in adipocytes (B. Omar et al., 2009). It is uncertain how PDE3B protein levels and activity increases by protein–
protein interaction with ABHD15. Though, suppressing the lipolysis of adipocyte in reaction to insulin hABHD15 appears to play a critical role. In white adipose tissue of mice high level expression of ABHD15 in vivo proposes the essentiality of ABHD15 in adipose tissues. Both the ABHD proteins ABHD5 and ABHD15 are extremely available in adipocytes and concerned in the lipolysis. Additional experiments will be essential to conclude how hABHD15 controls the nutrient metabolism and insulin action.

2.18. hABHD16A potential regulator of immunity

hABHD16A is a 558 amino acid residue protein of 63 kDa, formally identified as BAT5 (Human Lymphocyte Antigen B-associated transcript 5). hABHD16A is having both esterase hydrolase and acyltransferase domains. In mouse, mRNA of ABHD16A is universally expressed with maximum expression in brain. It have been documented that hABHD16A is palmitoylated enzyme (B.R. Martin et al., 2009). It was identified that hABHD16A is serine hydrolase by activity based protein profiling but true biochemical substrates and physiological function of hABHD16A are unidentified so far (H.S. Hoover et al., 2008). hABHD16A is component within cluster of genes MHC-III, signifying hABHD16A might be composite within immune resistance (T. Spies et al., 1989, T. Spies et al., 1989). In the development of autoimmune disease of vascular inflammation called Kawasaki disease the polymorphisms of hABHD16A are drastically connected (T. Spies et al., 1989). These polymorphisms in hABHD16A have been linked with back fat thickness of great white Italian pigs (Y.Y. Hsieh et al., 2010). Detection of the biochemical substrate specificity and function of hABHD16A could shed light on the supposed hABHD16A activity in immune system.

2.19. hABHD16B with unknown function

hABHD16B is located on chromosome 20q13.33 and is protein of 53 kDa. Subcellular position of ABHD16B is at present unknown. hABHD16B is not having acyltransferase domain however it is having a catalytic triad which might be responsible for esterase activity. There is no documented data available about the regulation of hABHD16B's mRNA, substrate specificity and function of hABHD16B is unidentified till to date.

2.20. Saccharomyces cerevisiae ABHD family enzymes and their functions

Many hydrolytic enzymes possessing multiple domains with diverse substrate specificities are identified from S.cerevisiae. Some of these enzymes are known orthologs of mammalian...
ABHD family lipases while the others are found to be redundant in *S. cerevisiae* (Karlheinz Grillitsch *et al.*, 2011). Tgl3p was the first TAG lipase of *Saccharomyces cerevisiae* reported to have conserved GXGXG lipase motif and a well conserved HXXXXD motif attributing acyl transferase activity. Tgl3p was found to be localised to lipid droplets and exhibited DAG hydrolysis activity along with definite lysophosphotidylethanolamine (LPE) Acyl transferase activity *in vitro* (Athenstaedt, K., and Daum, G *et al.*, 2003, Rajakumari, S., and Daum, G, 2010, Schmidt, C *et al.*, 2013). As will be talked about beneath, be that as it may, the lipolytic action of yeast TAG lipases isn't principal for their part in sporulation. Following this, Tgl4p and Tgl5p were reported with no trans-membrane domain but having the conserved lipase motif (Athenstaedt, K., and Daum, G, 2005).

Tgl4p is the major multifunctional enzyme involved in lipid metabolism. Along with Tgl3p, Tgl5p also displayed HXXXXD motif but preferentially acted as lysophosphatidicacid (LPA) Acyl tranferase. Both GXGXG and HXXXXD motifs were found to be acting independently. Mutational studies in lipase motif did not affect the Acyl transferase activity of Tgl5p (Rajakumari, S., and Daum, G, 2010). The other three yeast enzymes Yeh1, Yeh2 and Tgl1 constitute another class of membrane anchored lipases and contribute to steryl ester hydrolysis *in vivo* (Anita Jandrositz *et al.*, 2005, Köffel, R *et al.*, 2005, Bruno L. Bozaquel-Morais *et al.*, 2010). All these identified enzymes were found to be localized, either in LDs (Athenstaedt and Daum, 2003; Jandrositz *et al.*, 2005; Thoms *et al.*, 2011), mitochondria (Ham *et al.*, 2010), peroxisomes (Debelyy *et al.*, 2011; Thoms *et al.*, 2008), or vacuoles (Epple *et al.*, 2001; Teter *et al.*, 2001). In addition, the screening of knock out strains with different enzyme combinations still showed low, but persistent, TAG mobilization (Ploier *et al.*, 2013). The only characteristic property shared by all the known lipases is the presence of a conserved motif GXGXG in the active site with serine playing a vital role in catalysis. This catalytic triad is also found in an alpha beta hydrolase domain (ABHD) containing enzymes (Brady *et al.*, 1990; Kumar *et al.*, 2016; Schrag and Cygler, 1997; Wisotzkey *et al.*, 2003). LD hydrolase Ldh1p also had a GXGXG motif and was found to exert TAG lipase and esterase activities. *Ldh1Δ* showed the accumulation of nonpolar and phospholipids in lipid droplets (Debelyy *et al.*, 2011)

### 2.21. *Saccharomyces cerevisiae* as a model system

TAG and SE hydrolyzing enzymes are highly conserved across the phyla and *Saccharomyces cerevisiae* being a reliable model system, provided a platform for screening of such
hydrolyzing enzymes. From previous literature, the proliferating human cancer cell physiology and metabolic fluxes are very similar to those in the fermenting and rapidly proliferating yeast, which makes us to opt this eukaryote as an excellent experimental model for this study. Recent findings provides ample evidence that Lipases are the enzymes involved in lipid metabolism and were also found to play a major role in cancer cell proliferation. Human ABHD2 is expressed in breast and lung cancers but its biochemical functionality and substrate specificity was not clearly depicted. Similarly YMR210w and YPR147c were also reported to be involved in lipid metabolism, all these three enzymes belong to ABHD family.

The current study in the thesis investigates the role of human ABHD2, YMR210w and YPR147c in lipid metabolism and deals with their biochemical functionality and substrate specificity, the work flow and the objectives designed for the present study are given below.

2.22. AIM AND SCOPE OF THE WORK

To characterize Alpha Beta Hydrolase Domain family enzymes human ABHD2, its *Saccharomyces cerevisiae* functional orthologue YMR210w, and other ABHD family gene YPR147c, identifying their role in lipid metabolism.

2.23. OBJECTIVES

- Molecular Characterization of Human ABHD2 and its yeast functional ortholog YMR210w using *Saccharomyces cerevisiae* as a model System.
- Functional Characterization of *Saccharomyces cerevisiae*’s lipid droplet associated ABHD family enzyme YPR147c.
- Analysis of lipid profiles of wild type, ortholog deletion and over expressed strains of Human ABHD2, YMR210w and YPR147c genes.
WORK FLOW

Selection of genes (hABHD2, Ymr210w and Ypr147c) → Cloning into pYES2/CT vector individually → Transformation into DH5α, WT and Knock-Out strains of Saccharomyces cerevisiae (BY4741) → Optimizing the expression in yeast system →

- 6X His-Tag Protein purification
- Homology Modelling
- TEM Confocal Microscopy (WT, Δ, OΔ, Δ+)
- Growth Curve Analysis (YPR147c)
- Enzyme characterization (hABHD2, Ymr210w and Ypr147c)
- Molecular Docking studies (hABHD2)
- GC- MS analysis of lipid profile (WT, Δ)