International Journal of Scientific Research in Biological Sciences
Vol.5, Issue.6, pp.15-19, December (2018)
Research Paper
E-ISSN: 2347-7520

Lack of *FSH2* alters phospholipid metabolism in *Saccharomyces cerevisiae*

Ramachandran Gowsalya[1], Vasanthi Nachiappan[1]*

[1]Department of Biochemistry, School of Life Sciences, Bharathidasan University, Tiruchirappalli – 620 024, Tamil Nadu, India

**Corresponding author: vasanthibch@gmail.com, Mobile No. +91- 7639477488*

Available online at: www.isroset.org

Received: 06/Dec/2018, Accepted: 26/Dec/2018, Online: 31/Dec/2018

Abstract: Alpha/beta hydrolase domain containing protein act as a key regulator of lipid metabolism is extensively studied in *Saccharomyces cerevisiae*. However, the function of *FSH2* in lipid metabolism is still elusive, and we tried to understand the biological importance of *FSH2* in *Saccharomyces cerevisiae*. In our study, a protein containing alpha/beta hydrolase domain and belonging to the family of serine hydrolase (FSH) also possessed motifs of lipase (GXSXG) and acyl transferase (HX4D) that were identified using *in silico* analysis. The overexpression of *FSH2* in WT and *fsh2Δ* cells reduced the level of phospholipids, but in *fsh2Δ* strain increase in the cellular phospholipids, and this could be attributed to the formation of clumped aggregates and plasma membrane alteration were depicted using lipophilic fluorescent dye DiOC6. The current study suggested that the deletion of *FSH2* altered the phospholipid homeostasis and membrane morphology .Together, our results shown that the *FSH2* has a role in the phospholipid metabolism in *Saccharomyces cerevisiae.*

Keywords: Family of serine hydrolase, Lipid droplet, Phospholipid, αβ hydrolase domain.

I. INTRODUCTION

Lipids are a group of organic compounds that have extended attention due to the involvement in health and disease [1]. Membrane lipids are the key molecules that contribute in the structural component of cells and participate in the regulation of cellular processes such as signaling, energy supply, and cell death [2]. In the membranes of *S. cerevisiae*, the major phospholipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS), which are derived from the precursor lipid, phosphatidic acid (PA) [3, 4]. The PA is a central precursor for phospholipids and neutral lipids in *S. cerevisiae* [5]. In the CDP-DAG pathway, PE is synthesized by PS decarboxylase encoded by *PSD1* and *PSD2*. In yeast, the predominant PS decarboxylase is the Psd1p and is in the inner mitochondrial membrane, whereas the Psd2p is associated with Golgi/vacuole [6, 7, and 8]. The PE is further converted into PC by three consecutive methylation reactions, and the first step is catalyzed by *CHO2* (PE methylase) and, the next two methylation reactions are catalyzed by *OPI3* that results in PC [9, 10]. In the Kennedy pathway, PE and PC are synthesized, from exogenous ethanolamine and choline. The PC synthesis from choline is via the reaction catalyzed by *CKI1*-choline kinase, *PCT1*-choline-P cytidylyltransferase, and *CPT1*-choline phosphotransferase. The PC is a major phospholipid in all the organelles in *S. cerevisiae*, and it also serves as a signaling molecule that is involved in intercellular trafficking and apoptosis [11-15]. PE is the second major component of cell membrane and is important in the mitochondrial membranes and in involved in growth [15, 16].

In the present study, a yeast *FSH2* protein (uncharacterized protein) belonging to the family of serine hydrolases possessed the lipase motif (GXSXG) [17, 18], and acyl transferase (HX4D) domain. *In vitro* experiments suggested that the deletion of *fsh1* altered the cellular phospholipids, and this could be attributed to the plasma membrane alteration. Overexpression of the protein led to substantial reduction in total phospholipids. Collectively, these results elucidated that *FSH2* play an important role in phospholipid metabolism.

II. MATERIALS AND METHODS

Chemicals and media

Yeast extract, peptone, bacteriological agar were purchased from Hi-Media and yeast nitrogen base (YNB) from Difco. Thin-layer Silica GelF_{60} plates were purchased from Merck (India). Ampicillin, yeast synthetic drop-out medium and Nile red were purchased from Sigma.

Yeast strains, growth and media

The wild-type cells from *S. cerevisiae* BY4741 (*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*) and the genotypic

mutant *fsh2* were obtained as a gift from Prof. Ram Rajasekharan (Director, Lipidomic Center, Central Food Technological Research Institute (CFTRI**)**, India who obtained the stock from Euroscarf. Yeast cells were grown in YPD (1% yeast extract, 2% peptone, 2% dextrose)/defined synthetic medium at 30 °C on a rotary shaker. The yeast transformants (expression vector BG1805 - *FSH2* induced using galactose) carrying the expression plasmids were selected with uracil-free media. The transformed strains were grown in synthetic define medium and induced with 2% galactose.

Lipid extraction

Cells were grown until late log phase in 5 mL of SD-U containing 2% glucose. The cells were normalized and 0.1 OD ($A_{600\ nm}$) of cells were inoculated in a fresh medium containing 2% galactose and grown for 24 h. The cells were harvested and the lipids extracted [19]. The phospholipids were separated using chloroform/methanol/acetic acid (13:5:.1.6, v/v). The TLC plates was exposed to iodine, and the individual lipid spots scraped off, and the phosphorous was used to quantify the phospholipids.

Phosphorous estimation of phospholipids

Lipid samples were transferred into clean glass tubes and the solvent completely evaporated. To this 0.65 ml of perchloric acid was added and the tubes placed in the heated block for about 30 min or until the yellow color disappears. Silica gel spots are also digested in a similar manner. The tubes were allowed to cool, and 3.3 ml water, 0.5 ml of 2.5% molybdate solution and then 0.5 ml of 10% ascorbic acid were added and mixed using a vortex. The tubes were placed in a boiling water bath for 5 min and the tubes cooled and the absorbance of samples (including the standards) were read at $A_{800\ nm}$ and the standards (1 to 5 µg P/tube) were diluted with 3.3 ml water and 0.65 ml perchloric acid. Digestion is not necessary for standards before adding reagents. In the presence of silica gel (scraped from TLC plates), the tubes were centrifuged at low speed and its absorbance measured.

DiOC6 Membrane staining

DiOC6 staining. (3, 3′-dihexyloxacarbocyanine iodide) Wild-type, deletion cells were grown in SC medium at 30 °C, and cells were harvested at stationary phase. Cells were washed, re-suspended in TE buffer, and the cells were stained using 1.0 µl of the lipophilic dye DiOC6 (1 mg/ml ethanol). The fluorescence imaging was performed using an excitation at 488 nm and emission at 520 nm.

Statistical analysis

Results were expressed at mean ± standard deviation (SD) using graph pad prism version 6.0. Statistical analysis was carried out by Student's t test or two way ANOVA. Each experiment was repeated minimum of three times. Significance was determined at *p<0.05.

III. RESULTS AND DISCUSSION

Fsh2p belongs to the αβ hydrolase family

In yeast, many genes are yet to be identified for their function. In the present study, we examined the functional importance of *FSH2* in yeast lipid metabolism. The *FSH2* possessed a αβ hydrolase domain. The gene, possessed a highly conserved stretch of amino acids ^{4} NXXXXH ^{9} and ^{108}GXSXG^{112} (Fig. 1A and B), and the majority of known lipases, phospholipases, lysophospholipases, esterases, serine proteases [20], and acyl transferases .Knowing this aspect BLAST analysis was performed and we tried to determine the Fsh2p homologues among prokaryotes and eukaryotes. The Fsh2p possessed more than 20% sequence similarity with mammalian gene *OVCA2* and other plants [17]. The hydropathy plot analysis predicted the protein to possess a transmembrane helix, and it is highly associated with the cytosol (Fig. 1C).

A

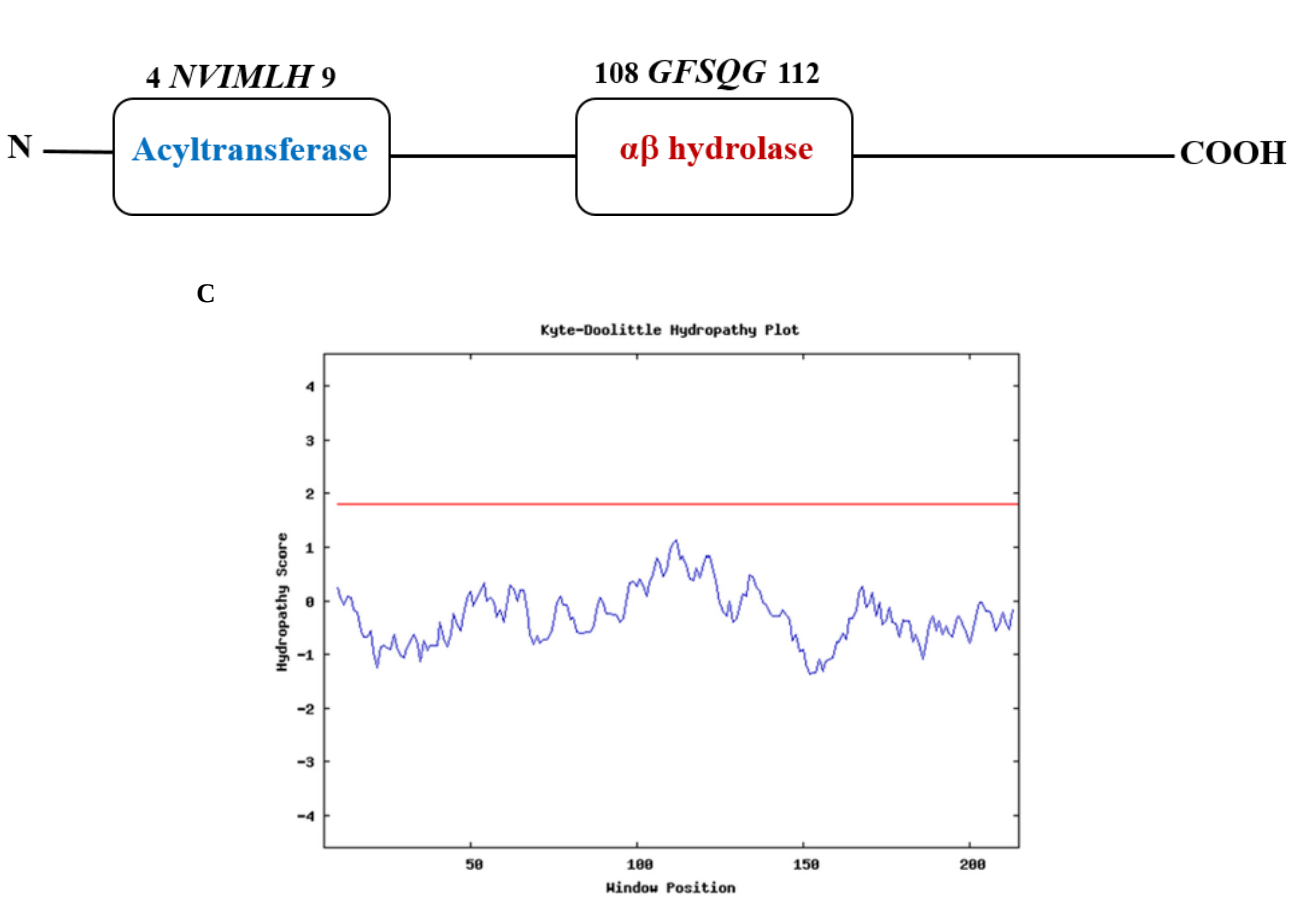
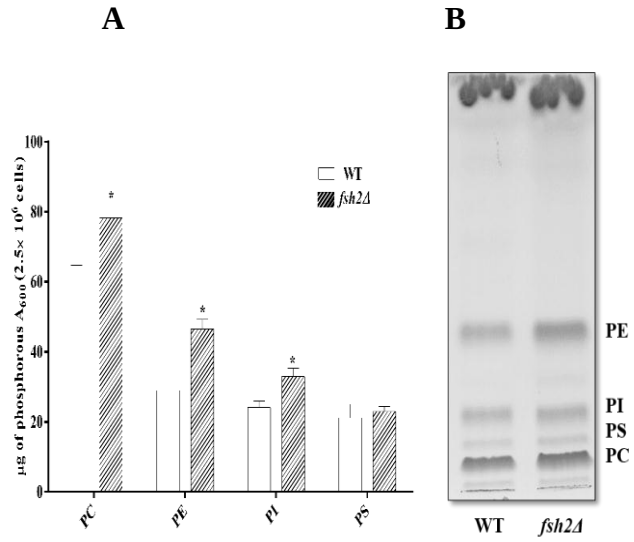


Fig. 1 Structural prediction of *FSH2*. (A) The motif of *FSH2* was determined by EXPASY PROSITE analysis and is indicated to possess both lipase and acyltransferase motif. (B) Schematic representation of αβ hydrolase domain**.** (C) Hydropathy plot was performed for Fsh2p using hydropathy plot. Peaks with scores greater than 1.8 (red line) indicate possible transmembrane protein and below 1.8 indicate a possible cytosolic protein.

Family of serine hydrolase 2 defect leads to accumulate membrane lipids

The *in-silico* analysis predicted that the protein might involve in lipid metabolism, since it possessed lipase motif. Here in yeast we analyzed the role of *FSH2* (family of serine hydrolase 2) in lipid metabolism (phospholipids). In yeast during exponential phase phospholipid is important for the membrane maintenance and cell viability [15, 21 and 22]. When compared to the WT cells, the *fsh2*Δ cells significantly increased the major phospholipids PC, PE and the minor phospholipid PI (Fig. 2A and B). The deletion of *FSH2* increase the proliferation of intercellular membrane and increase the solid green fluorescence throughout the cells, and this could be attributed to the alteration of plasma membrane compared with WT cells (Fig. 2C). These results suggested that *FSH2* is involved in the phospholipid metabolism and it's important for the membrane morphology in yeast.



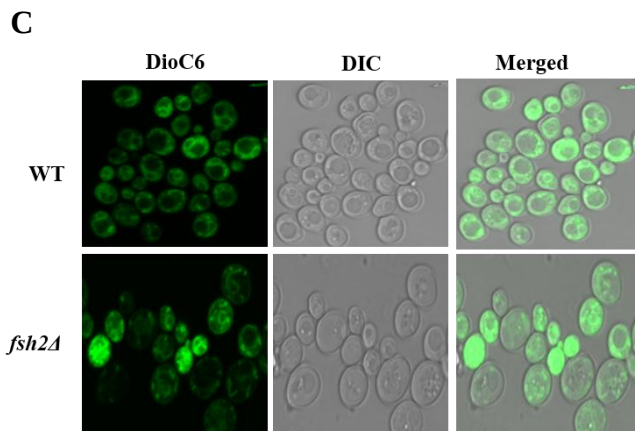


Fig. 2 Analysis of phospholipid in WT and *fsh2Δ*. (A) Cells were grown in synthetic defined medium in presence of glucose as a carbon source up to the stationary phase and an equal amount ($A_{600\ nm}$ = 50) of cells were used for lipid extraction. Phospholipids were resolved on TLC using solvent systems, chloroform/methanol/acetic acid (13:5:1.6, v/v). (B) The amount of phosphorous present in the cells was quantified and each value represents the mean ± SD of three independent experiments. (C) Membrane staining by DiOC6. The WT and *fsh2Δ* cells were grown in SC up to mid log phase, and the cells were stained with the DiOC6 lipophilic dye and the fluorescence imaging was performed on confocal microscope.

Over expression of *FSH2* in WT and *fsh2Δ* stain decreased the cellular phospholipids

The wild and *fsh2Δ* strains were transformed with yeast expression vectors (both BG1805 and BG1805-*FSH2*). The transformed colonies were grown in the presence of synthetic defined media, without uracil (SD -U) and galactose as a carbon source and the cells were grown for 24 h. Fsh2p mutant increases the phospholipids and over-expression of *FSH2* decreased PC, PE, and PI decreased (Fig. 3A and B).These results suggested that overexpression of *FSH2* reduced the lipid content in yeast.

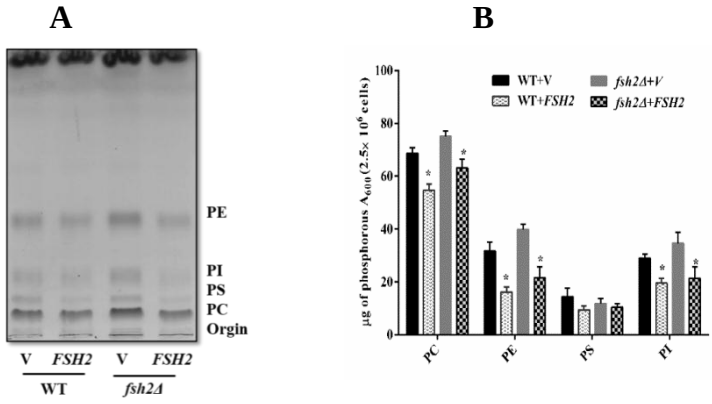


Fig. 3 Analysis of phospholipid in *FSH2* overexpressed cells. (A) Yeast cells were overexpressed with *FSH2* or the vector control and were grown for 24 h in galactose medium. An equal number of cells (50 OD at $A_{600\ nm}$) were taken and the lipids extracted. The phospholipids were separated by silica TLC using solvent systems, chloroform/methanol/acetic acid (13:5:1.6, v/v). Phosphatidylinositol (PI); phosphatidylserine (PS); phosphatidylcholine (PC); phosphatidylethanolamine (PE). (B) The amount of phospholipid present in the cells was quantified by the phosphorous estimation and each value represents the mean ± SD of three independent experiments.

VI. CONCLUSION

The deletion of *FSH2* shown to increase the cellular PC and PE level compared to WT cells. On the other hand, the over expression of *FSH2* decreases the PC and PE level in control strain, Hence, the *in silico* analysis also supported that the *FSH2* possess lipase and acyltransferase motif, so *in vitro* experiments evidenced that the deletion of *fsh2* increase the cellular phospholipids, and this could be attributed to the plasma membrane alteration. Finally, we conclude that *FSH2* important for the maintenance of phospholipid homeostasis. This is the first report for the existence of *FSH2* plays an important role in phospholipid metabolism under physiological conditions. Further work is needs to study the molecular mechanism and regulation of *FSH2* in lipid metabolism.

V. ACKNOWLEDGMENTS

We thank Prof. Ram Rajasekharan (Central Food Technological Research Institute, Mysore, India) for providing yeast strains. We thank the infrastructure facilities from DST-FIST, Department of Biochemistry, Life Sciences & DST-PURSE facilities of Bharathidasan University.

REFERENCE

[1]. L. Klug, G. Daum, "*Yeast lipid metabolism at a glance*", FEMS Yeast Res, Vol.14, Issue.3, pp 369–88, 2014.

[2]. M. George, Carman and Geri Marie Zeimetz, "*Regulation of Phospholipid Biosynthesis in the Yeast Saccharomyces cerevisiae and interrelationship with other metabolic processes*", Prog. Lipid Res, Vol. 38, Issue.5-6, pp 361-399, 1996.

[3]. S.A. Henry , S.D. Kohlwein , G.M. Carman , "*Metabolism and Regulation of Glycerolipids in the Yeast Saccharomyces cerevisiae*", Genetics, Vol.190, Issue.2, pp 317-349, 2012.

[4]. H.J. Kaiser , M.A. Surma , F. Mayer , I. Levental , M. Grzybek , R.W. Klemm , S. Da Cruz , C. Meisinger , V. Müller , K. Simons , D. Lingwood , "*Molecular convergence of bacterial and eukaryotic surface order*", J. Biol. Chem, Vol.286, Issue.47, pp 40631–40637, 2011.

[5]. R. Nebauer, R. Birner-Grünberger, G. Daum, "*Biogenesis and cellular dynamics of glycerophospholipids in the yeast Saccharomyces cerevisiae*", Lipid Metabolism and Membrane Biogenesis Genet, Vol. 6, pp125–168, 2003.

[6]. G.M. Carman and G.S. Han, "*Regulation of phospholipid synthesis in the yeast Saccharomyces cerevisiae*", Annu Rev Biochem, Vol.80, pp 859–883, 2011.

[7]. C.J. Clancey, S.C. Chang, W. Dowhan, "*Cloning of a gene (PSD1) encoding phosphatidylserine decarboxylase from Saccharomyces cerevisiae by complementation of an Escherichia coli mutant*", J Biol Chem, Vol. 268, pp 24580–24590, 1993.

[8]. P.J. Trotter and D.R. Voelker, "*Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (PSD2) in the yeast Saccharomyces cerevisiae*", J Biol Chem, Vol. 270, Issue.11, pp 6062–6070, 1995.

[9]. E.F. Summers, V.A. Letts, P. McGraw and S.A. Henry, "*Saccharomyces cerevisiae cho2 mutants are deficient in phospholipid methylation and cross-pathway regulation of inositol synthesis*", Genetics, Vol.120, Issue.4, pp 909–922, 1988.

[10]. P. McGraw and S.A. Henry, "*Mutations in the Saccharomyces cerevisiae opi3 gene: effects on phospholipid methylation, growth and cross-pathway regulation of inositol synthesis*", Genetics, Vol.122, Issue.2, pp 317–330, 1989.

[11]. J.H. Exton, "*Phosphatidylcholine break down and signal transduction*", Biochim. Biophys. Acta, Vol. 1212, Issue.1, pp 26–42, 1994.

[12]. C. Kent, G.M. Carman, "*Interactions among pathways for phosphatidylcholine metabolism, CTP synthesis and secretion through the Golgi apparatus*", Trends BiolChem. Sci, Vol.24, Issue.4, pp 146–150, 1999.

[13]. Z. Cui, M. Houweling, "*Phosphatidylcholine and cell death*", Biochim. Biophys. Acta, Vol.1585, Issue.2-3, pp 87–96, 2002.

[14]. G. Howe, C.R. McMaster, "*Regulation of vesicle trafficking, transcription, and meiosis: lessons learned from yeast regarding the disparate biologies of phosphatidylcholine*", Biochim. Biophys. Acta, Vol.1534, Issue.2-3, pp 65–77, 2001.

[15]. A.W. James, R. Gowsalya, V. Nachiappan , "*Dolichyl pyrophosphate phosphatase-mediated N-glycosylation defect dysregulates lipid homeostasis in Saccharomyces cerevisiae*", Biochim. Biophys. Acta, Vol.1861, Issue.11, pp 1705-1718, 2016**.**

[16]. R. Birner, M. Burgermeister, R. Schneiter, G. Daum, "*Roles of phosphatidylethanolamine and of its several biosynthetic pathways in Saccharomyces cerevisiae*", Mol. Biol. Cell, Vol.12, Issue.4, pp 997–1007, 2001.

[17]. S.M. Baxter, J.S. Rosenblum, S. Knutson, M.R. Nelson, J.S. Montimurro, J.A. Di Gennaro, J.A. Speir, J.J. Burbaum, J.S. Fetrow, "*Synergistic computational and experimental proteomics approaches for more accurate detection of active serine hydrolases in yeast*", Mol. Cell. Proteomics, Vol.3, Issue.3, pp 209-25, 2004.

[18]. S. Quevillon-Cheruel, N. Leulliot, M. Graille, N. Hervouet, F. Coste, H. Bénédetti, C. Zelwer, J. Janin , H. Van Tilbeurgh, "*Crystal structure of yeast YHR049W/FSH1, a member of the serine hydrolase family, Protein", Sci* , Vol.14, Issue.5, pp 1350–1356, 2005.

[19]. E.G. Bligh and W.J. Dyer, "*A rapid method of total lipid extraction and purification*", J. Biochem. Physiol Vol.37, Issue.8, pp 911-917, 1959.

[20]. Y. Wei, J.A. Contreras, P. Sheffield, T. Osterlund, U Derewenda, Kneusel R.E, Matern U, Holm C, Zygmunt S "*Crystal structure of brefeldin A esterase, a bacterial homolog of the mammalian hormone-sensitive lipase*", Nat. Struct. Biol, Vol.6, Issue.4, pp 6:340–345, 1999.

[21]. G.M. Carman, S.A. Henry, "*Phospholipid biosynthesis in the yeast Saccharomyces cerevisiae and interrelationship with other metabolic processes*", Prog. Lipid Res, Vol.38, Issue.5-6, pp 361–399, 1999.

[22]. S.E. Horvath, A. Wagner, E. Steyrer, G. Daum, "*Metabolic link between phosphatidylethanolamine and triacylglycerol metabolism in the yeast Saccharomyces cerevisiae",* Biochim. Biophys. Acta, Vol.1811, Issue 12, pp 1030-7, 2011.

