Form for Submission of Research Proposal using Stem Cells for Approval by Institutional Committee on Stem Cell Research and Therapy (IC-SCRT)

1. Project number (to be assigned by IC-SCRT):

2. Title of the Proposal: Mechanisms and functional consequences of compositional regulation at the local and global scale in membranes of living cells Sub title: 1. Calibration of insulin receptor signaling via regulation of local membrane lipid composition 2. Role of membrane microdomains in insulin receptor signaling and obesity linked insulin resistance

3. Name of the applicant/s with designation and qualifications:

Particulars	**Name, Designation & Qualification**	**Contact Address & Telephone No / Mobile No and e-mail ID**	**Signature**
Principal Investigator	Satyajit Mayor Professor PhD	National Centre for Biological Sciences, Bangalore-560065 Phone: +91 80 23666260 E mail: mayor@ncbs.res.in	
Co-PI / Co-investigator			
1	Dr. Arpita Mukhopadhyay Assistant Professor PhD	Division of Nutrition, St. John's Research Institute, Bangalore-560034 Mobile no: 9742802746 Email: arpitam@sjri.res.in	
2	Dr. Anura V Kurpad Head, Division of Nutrition & Department of Nutrition MBBS,MD, PhD	Division of Nutrition, St. John's Research Institute, Bangalore-560034 Mobile no: 9686512233 Email: a.kurpad@sjri.res.in	
3	Dr. Sridar Govindaraj Professor and Head, Department of General Surgery MBBS, MS, DNB, FRCS	Department of General Surgery, St. John’s Medical College and Hospital, Bangalore-560034 Mobile no: 9845366846 Email: sridar_sasi@yahoo.com	
4	Dr. Anupama Ambika Anilkumar, Post Doctoral Fellow PhD	Division of Nutrition, St. John's Research Institute, Bangalore-560034 Mobile No: 7829778188 Email: ambikaa@ncbs.res.in	
Co-ordinators			

1	Dr. Anupama Ambika Anilkumar, Post Doctoral Fellow PhD	Division of Nutrition, St. John's Research Institute, Bangalore-560034 Mobile No: 7829778188 Email: ambikaa@ncbs.res.in	

4. Multi-institutional study ? ☑ Yes ☐ No

5. Briefly outline the work done previously in the field with relevant references: (Introduction, review of literature, justification for study, highlighting the need for the study, potential risks and benefits and outcome measures). (not more than 1-2 pages)

Introduction & Review of literature The fluid mosaic model proposed in early 1970's by Singer and Nicholson (Singer and Nicolson, 1972) considered the structure of the plasma membrane of a living cell as a mosaic of different phospholipids, proteins and cholesterol. This simple model was purely based on equilibrium thermodynamics of lipid protein interactions and treated lipids as solvents for the membrane proteins which indicated towards the non-existence of local lateral heterogeneities at the plasma membrane. The current idea of the plasma membrane has evolved into a complex picture where the multitude of lipids and proteins are compartmentalized into microdomains at the cell surface for the efficient functioning of the plethora of membrane proteins and the cell in general. The local membrane organization of these lipids and proteins are finely tuned and are known to be far from thermal equilibrium. For the efficient functioning of the cellular proteins the domain composition has to be actively regulated. Recent work has shown the pivotal role of the cortical actin cytoskeleton in regulating the generation of these membrane domains (Goswami et al., 2008; Gowrishankar et al., 2012). This process is facilitated via a transbilayer coupling mechanism mediated by the inner leaflet lipid, phosphatidylserine (PS) which has the capacity to engage with the actin cytoskeleton via its interaction with several actin binding adaptor proteins (Raghupathy et al., 2015). Experimental evidences have shown that the fine-tuning of the local lipid composition helps in the generation and maintenance of such microdomains which could have functional consequences at multiple scales. By exploring the transbilayer coupling mechanism in greater detail, it has been observed that the lipid chemistry of the outer leaflet proteins/lipids and their inner leaflet counterparts along with sufficient membrane cholesterol are pre-requisites for generation of such domains possessing the characteristics of a liquid ordered (lo) phase. Ongoing work has further provided evidences for receptor molecules (particularly in the context of integrin signaling) altering the local lipid composition of the membrane thereby helping the formation/fragmentation of membrane domains which would serve as membrane platforms for the recruitment of their downstream signaling molecules. Similarly, insulin receptor signaling in the context of altered local membrane composition is an interesting example to study how the local membrane niche serves to fine tune the activation of the insulin signaling cascade itself. A number of studies carried out on insulin receptor signaling have shown that the localization of the insulin receptor to membrane domains enriched in cholesterol is indispensable for the activation of the insulin signaling cascade and relocalization of these receptor from such microdomains ultimately leads to the dysfunction of the receptor itself (Czech, 2000; Morino-Koga et al., 2013; Sánchez-Wandelmer et al., 2009; Vainio et al., 2002, 2005). The lipid composition of the membrane is maintained and regulated by several intrinsic and extrinsic factors. The extrinsic factors include majorly the dietary source of lipids which could exert a significant influence on the overall lipid composition of the membrane. The exogenously incorporated fatty acids could be efficiently translated into the desired lipid species by the action of several lipid remodeling enzymes and acyl transferases resulting in acute alteration of the plasma membrane lipid composition. This strategy could be adopted to explore the how the local lipid environment

calibrates the efficient functioning of the insulin receptor. To test this hypothesis, influence of dietary lipid composition and associated epigenetic (DNA methylation) changes on insulin sensitivity and glucose uptake in the context of type 2 diabetes will be tested. This study will be conducted on primary adipocytes procured from insulin resistant as well as normoglycemic individuals. This will help in understanding the influence of modulation of membrane lipid composition and related modulation of DNA methylation in the activation of insulin signaling pathway and will throw some light on how one could overcome insulin resistance by simply fine tuning the local lipid composition. Justification / need for the study The lipid composition of the plasma membrane is maintained and regulated by several external and internal factors. The heterogeneity which exists at the level of the plasma membrane is the result of the generation of membrane domains at the cell surface. These domains serve as reaction centers for the activation of a number of cell surface receptors like the insulin receptor. Understanding how the local membrane niche helps in the activation of this receptor will provide us with valuable insights into the role of membrane composition in regulating insulin receptor activation. Moreover information regarding the external inputs for example the effect of dietary lipids in the local membrane organization will help us understand how the cell compartmentalize various cellular components to assist in the efficient functioning of the insulin receptor. Therefore it is imperative to have a model system that will help manipulate the plasma membrane lipid composition either genetically or by exogenous addition of dietary lipids. The current study aim to test the hypothesis that dietary lipids affect insulin sensitivity and glucose uptake by regulating DNA methylation and the insulin receptor signaling pathway, especially in primary adipose tissues. This would help in analyzing the insulin dependent membrane organization capacity. Accomplishing these objectives will provide with valuable insights into how receptor activation assists in the self-organization of the plasma membrane and vice versa. Several experimental approaches as well as theoretical framework suggest that the generation of membrane domains is brought about by the cortical actin cytoskeleton and they help in the maintenance of domain size and shape by actively engaging with several actin binding adaptors (Goswami et al., 2008; Gowrishankar et al., 2012; Raghupathy et al., 2015; Sharma et al., 2004). The coupling between the outer leaflet components residing in these domains and the actin cytoskeleton lying beneath the inner leaflet is mediated by the inner leaflet lipid phosphatidylserine via a transbilayer coupling mechanism (Raghupathy et al., 2015). It has been observed that the chemistry of the outer leaflet and the inner leaflet lipids is extremely important for generating these domains along with adequate amounts of cholesterol in either leaflet. Thus determining the molecular players that help in the building of these domains by linking the surface receptors to the membrane-actin machinery have prompted us to explore the functional consequences of the generation, maintenance and fragmentation of these domains. By perturbing the membrane domain formation by affecting the receptor's capacity to form such domains, the functional consequences could be potentially analyzed. To this end, assays needs to be designed which would enable systematic disruption of the membrane domains and look for the signaling output of a cell surface receptor like the insulin receptor. The adipocyte insulin receptor is known to be associated to caveolae containing 'lipid rafts' (Vainio et al., 2002). The autophosphorylation enabled by the receptor upon activation is compromised if such membrane domains are disrupted following membrane extraction of cholesterol. In hepatocytes where caveolae is absent the insulin receptor gets recruited to such membrane domains only upon activation. The localization of insulin receptor to these domains is indispensable for the progression of the insulin signaling cascade. It is known that membrane receptors are capable of modulating their local lipid environment favoring the formation of such membrane domains particularly in the context of integrin receptors. Mechanism of similar stature might be prevalent even in the context of insulin receptor signaling. Dietary intake of several fatty acids could be efficiently translated into the plasma membrane in the form of lipid components resulting in drastic alterations in the lipid composition across the membrane. This might also reflect changes in the micron scale properties of the lipid bilayer and associated changes in DNA methylation of critical loci in insulin signaling. Monitoring insulin receptor activation and progression of the signaling cascade in this context will help in understanding how and why the cell membrane calibrates its local membrane lipid composition to enhance

the efficacy of a particular process. To this end we are planning to analyse the membrane properties under conditions of insulin resistance in mice as well as human subjects who are insulin resistant. We plan to perform stem cell reprogramming and genome editing in adipocytes differentiated from the blood sample isolated from human subjects in order to identify the molecular mechanism underlying insulin resistance. References 1. Czech, M.P. (2000). Lipid rafts and insulin action. Nature 407, 147–148. 2. Goswami, D., Gowrishankar, K., Bilgrami, S., Ghosh, S., Raghupathy, R., Chadda, R., Vishwakarma, R., Rao, M., and Mayor, S. (2008). Nanoclusters of GPI-anchored proteins are formed by cortical actin-driven activity. Cell 135, 1085–1097. 3. Gowrishankar, K., Ghosh, S., Saha, S., C, R., Mayor, S., and Rao, M. (2012). Active remodeling of cortical actin regulates spatiotemporal organization of cell surface molecules. Cell 149, 1353–1367. 4. Morino-Koga, S., Yano, S., Kondo, T., Shimauchi, Y., Matsuyama, S., Okamoto, Y., Suico, M.A., Koga, T., Sato, T., Shuto, T., et al. (2013). Insulin receptor activation through its accumulation in lipid rafts by mild electrical stress. J. Cell. Physiol. 228, 439–446. 5. Raghupathy, R., Anilkumar, A.A., Polley, A., Singh, P.P., Yadav, M., Johnson, C., Suryawanshi, S., Saikam, V., Sawant, S.D., Panda, A., et al. (2015). Transbilayer Lipid Interactions Mediate Nanoclustering of Lipid-Anchored Proteins. Cell 161, 581–594. 6. Sánchez-Wandelmer, J., Dávalos, A., Herrera, E., Giera, M., Cano, S., de la Peña, G., Lasunción, M.A., and Busto, R. (2009). Inhibition of cholesterol biosynthesis disrupts lipid raft/caveolae and affects insulin receptor activation in 3T3-L1 preadipocytes. Biochim. Biophys. Acta 1788, 1731–1739. 7. Sharma, P., Varma, R., Sarasij, R.C., Ira, Gousset, K., Krishnamoorthy, G., Rao, M., and Mayor, S. (2004). Nanoscale organization of multiple GPI-anchored proteins in living cell membranes. Cell 116, 577–589. 8. Singer, S.J., and Nicolson, G.L. (1972). The fluid mosaic model of the structure of cell membranes. Science (80-.). 175, 720–731. 9. Vainio, S., Heino, S., Mansson, J.-E., Fredman, P., Kuismanen, E., Vaarala, O., and Ikonen, E. (2002). Dynamic association of human insulin receptor with lipid rafts in cells lacking caveolae. EMBO Rep. 3, 95–100. 10. Vainio, S., Bykov, I., Hermansson, M., Jokitalo, E., Somerharju, P., and Ikonen, E. (2005). Defective insulin receptor activation and altered lipid rafts in Niemann-Pick type C disease hepatocytes. Biochem. J. 391, 465–472.

6. Aims and Objective(s) of the current Study

• Understanding the change in the plasma membrane lipid composition under conditions of insulin activation • Determination of changes in plasma membrane lipid composition and associated DNA methylation changes of primary adipose tissues from patients who exhibit insulin resistance compared to normoglycaemic subjects • Regulation of plasma membrane lipid composition to understand the influence of the local membrane environment in the proper functioning of the cell surface insulin receptor

7. Funding Agency:

Wellcome Trust- DBT Margdarshi fellowship

8a. Project start date: 15-Nov-2017 **Project End date:** 15-Nov-2020

8b. Project status: New

9. Summary of research in layman's terms (200 words):

Plasma membrane of the cell is made up of lipid bilayer which is decorated with many proteins known as receptors which recognize specific molecules (ligands) and send messages (signal) to instruct the cell to perform specific function. For efficient functioning of membrane receptors these molecules along with membrane lipids needs to be organized in specialized structures (microdomains- MD) which initiates receptor signaling. My hypothesis is that any alteration in constituents of MD can influence receptor signaling by defective MD assembly. I will test this by studying insulin receptor (IR) signaling as an

experimental model. The IR binds to insulin and instructs cells to burn glucose. However, it is not clear whether defective MD assembly due to lipotoxicity leads to obesity associated insulin resistance. I will test this by using mouse models of obesity as well as human stem cells that are lacking specific lipid or protein components important for membrane MD assembly. For this I would require to obtain blood samples from human subjects, reprogram them into iPSCs, perform genome editing and then differentiate them into adipocytes to look at the link between obesity driven change in lipid composition and insulin resistance.

10. Stem cell details:

(i)Type of stem cell:

☐ Embryonic ☐ Fetal ☐ Adult ☑ iPSc

(ii)Model system:

☑ Human ☐ Mouse ☐ Rat ☐ Other

(iii)Mode of study:

☑ in vitro ☐ in vivo

(iv)Transplantation into model organisms:

☐ Yes ☑ No

(v)Human subjects:

☐ Yes ☑ No

11. If human cells/subjects are involved, IEC of human studies approved the proposal? ☐ Yes ☑ No

12. Other committee approvals from NCBS

IAEC approval: ☑ Yes ☐ No ☐ NA

IBSC approval: ☑ Yes ☐ No ☐ NA

IEC approval: ☐ Yes ☑ No ☐ NA

13.Approvals from other collaborating institute (if applicable)"(enclosures may include ethical approvals/Material Transfer Agreement (MTA)/ MoU)"

File attached

File Attachments :

Signature of PI:
Date: 15/11/2017

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3. Name of the applicant/s with designation and qualifications:

Particulars	**Name, Designation & Qualification**	**Contact Address & Telephone No / Mobile No and e-mail ID**	**Signature**
Principal Investigator	Prof. Satyajit Mayor Senior professor PhD	National Centre for Biological Sciences, Bangalore-560065 Phone: +91 80 23666260 E mail: mayor@ncbs.res.in	
Co-PI / Co-investigator			
1	Dr. Arpita Mukhopadhyay Assistant Professor PhD	Division of Nutrition, St. John's Research Institute, Bangalore-560034 Mobile no: 9742802746 Email: arpitam@sjri.res.in	
2	Dr. Anura V Kurpad Head, Division of Nutrition & Department of Nutrition MBBS,MD, PhD	Division of Nutrition, St. John's Research Institute, Bangalore-560034 Mobile no: 9686512233 Email: a.kurpad@sjri.res.in	
3	Dr. Sridar Govindaraj Professor and Head, Department of General Surgery MBBS, MS, DNB, FRCS	Department of General Surgery, St. John’s Medical College and Hospital, Bangalore-560034 Mobile no: 9845366846 Email: sridar_sasi@yahoo.com	
4	Anupama Ambika A Post Doctoral Fellow PhD	Division of Nutrition, St. John's Research Institute, Bangalore-560034 Mobile No: 7829778188 Email: ambikaa@ncbs.res.in	
Co-ordinators			

1	Anupama Ambika A Post Doctoral Fellow PhD	Division of Nutrition, St. John's Research Institute, Bangalore-560034 Mobile No: 7829778188 Email: ambikaa@ncbs.res.in	

4. Multi-institutional study ? ☑ Yes ☐ No

5. Briefly outline the work done previously in the field with relevant references: (Introduction, review of literature, justification for study, highlighting the need for the study, potential risks and benefits and outcome measures). (not more than 1-2 pages)

Introduction The fluid mosaic model proposed in early 1970's by Singer and Nicholson 1 considered the structure of the plasma membrane of a living cell as a mosaic of different phospholipids, proteins and cholesterol. The current idea of the plasma membrane has evolved into a complex picture where the multitude of lipids and proteins are compartmentalized into microdomains at the cell surface for the efficient functioning of the plethora of membrane proteins and the cell in general. The local membrane organization of these lipids and proteins are finely tuned and are known to be far from thermal equilibrium. For the efficient functioning of the cellular proteins the domain composition has to be actively regulated. Experimental evidences have shown that the fine-tuning of the local lipid composition helps in the generation and maintenance of such microdomains which could have functional consequences at multiple scales. Ongoing work has provided evidences for receptor molecules (particularly in the context of integrin signaling) altering the local lipid composition of the membrane thereby helping the formation/fragmentation of membrane domains which would serve as membrane platforms for the recruitment of their downstream signaling molecules. Similarly, insulin receptor signaling in the context of altered local membrane composition is an interesting example to study how the local membrane niche serves to fine tune the activation of the insulin signaling cascade itself. A number of studies carried out on insulin receptor signaling have shown that the localization of the insulin receptor to membrane domains enriched in cholesterol is indispensable for the activation of the insulin signaling cascade and relocalization of these receptor from such microdomains ultimately leads to the dysfunction of the receptor itself 2–6. To test this hypothesis, influence of dietary lipid composition and associated epigenetic (DNA methylation) changes on insulin sensitivity and glucose uptake in the context of type 2 diabetes will be tested. This study will be conducted on primary adipocytes procured from insulin resistant as well as normoglycemic individuals. This will help in understanding the influence of modulation of membrane lipid composition and related modulation of DNA methylation in the activation of insulin signaling pathway and will throw some light on how one could overcome insulin resistance by simply fine tuning the local lipid composition. Justification / need for the study Several experimental approaches as well as theoretical framework suggest that the generation of membrane domains is brought about by the cortical actin cytoskeleton and they help in the maintenance of domain size and shape by actively engaging with several actin binding adaptors 7–10. The coupling between the outer leaflet components residing in these domains and the actin cytoskeleton lying beneath the inner leaflet is mediated by the inner leaflet lipid phosphatidylserine via a transbilayer coupling mechanism 10. It has been observed that the chemistry of the outer leaflet and the inner leaflet lipids is extremely important for generating these domains along with adequate amounts of cholesterol in either leaflet. Thus determining the molecular players that help in the building of these domains by linking the surface receptors to the membrane-actin machinery have prompted us to explore the functional consequences of the generation, maintenance and fragmentation of these domains. By perturbing the membrane domain formation by affecting the receptor's capacity to form such domains, the functional consequences could be potentially analyzed. To this end, assays needs to be designed which would enable

systematic disruption of the membrane domains and look for the signaling output of a cell surface receptor like the insulin receptor. For this we intend to analyse the membrane properties under conditions of insulin resistance in human subjects who are insulin resistant. We plan to perform stem cell reprogramming and genome editing in adipocytes differentiated from the blood sample isolated from human subjects in order to identify the molecular mechanism underlying insulin resistance. For this purpose we prefer to use iPSCs compared to any other progenitors simply because of the ease of generation of iPSCs from blood samples as compared to mesenchymal stem cells obtained from bone marrow. We chose to use iPSCs compared to adipocytes as the model system for the following reasons: 1. Adipocytes are obtained from patients during laproscopic surgery while iPSCs are generated from blood samples which are easily obtained from patients and hence this method is less invasive 2. iPSCs are cell lines whereas adipocytes are primary cells hence the latter is tend to introduce more variability into the experiments performed since they are not isolated from a single clone. 3. Sustainability and renewability is more for iPSCs compared to primary adipocytes. Moreover, iPSCs will be obtained from PBMCs by using the protocol standardised in the lab of Toni-Vidal Puig (personal communication). Since this is an already established protocol of adipocyte generation we would prefer to use this as our ideal model system. The iPS cells are maintained on feeder layers of irradiated or Mitomycin C inactivated mouse embryonic fibroblasts or on Matrigel prior to directed differentiation to adipocytes. CRISPR mediated genome editing will beperformed on these adipocytes using oligonucleotides. We plan to knockout genes involved in cell cell adhesions (Vinculin) and in the fatty acid remodelling of lipid anchors (Pgap2 and PGAP3) to understand the mechanism of insulin resistance in great detail since insulin receptor signaling is known to be influenced by the local environment in which the receptor resides. In addition to this model system we also intend to carry out membrane organization and lipidomic profiling of primary adipocytes isolated from mouse strains which are made insulin resistant either by genetic manipulation or by subjecting them to high fat diet. Proposed Methodology/protocol To fulfill the objectives of this study, it is planned to recruit diabetic and normoglycaemic normal BMI and overweight male and female subjects. The subjects for this study will be recruited at the Department of General Surgery, St. John’s Medical College and Hospital, Bangalore. (i) • Inclusion Criteria 1. Subjects of normal BMI (?18.5 kg/m2 and <25.0 kg/m2) and overweight BMI (?25.0 kg/m2 and <30.0 kg/m2) (15 males and 15 females in each group; age: 25-50 years) 2. Age: 25-50 years 3. Type-2 Diabetics (for the case group: 30 subjects with normal BMI and 30 subjects with overweight BMI) and normoglycemic subjects (for the control group: 30 subjects with normal BMI and 30 subjects with overweight BMI) • Exclusion Criteria 1. Age outside the range of 25-50 years. 2. BMI <18.5 kg/m2 (underweight) or ?30.0 kg/m2 (obese). 3. Individual who have undergone surgeries in the last 6 months. 4. Individuals who are participating in weight loss programs. 5. Those participating in any other study and those who tested positive for hepatitis (HBsAg), HIV or syphilis (VDRL) infections. Those who have serious pre-existing medical condition will be excluded, and these will be defined as conditions that require chronic or daily medical therapy. Examples include connective tissue diseases, hypertension, inflammatory bowel disease, active tuberculosis, symptomatic heart disease. (ii) Type of subjects: No. of Males and/or Females: 60 males and 60 females Volunteers : ?Yes / No Patients : ?Yes / No Vulnerable subjects : Yes / No? In the proposed methodology, only blood, urine and adipose tissue samples will be collected. The adipose tissue samples will be collected during laparoscopic surgery procedure that the subjects have been advised to undergo by their attending clinician. There is no intervention planned. References 1. Singer, S. J. & Nicolson, G. L. The fluid mosaic model of the structure of cell membranes. Science (80-.). 175, 720–31 (1972). 2. Sánchez-Wandelmer, J. et al. Inhibition of cholesterol biosynthesis disrupts lipid raft/caveolae and affects insulin receptor activation in 3T3-L1 preadipocytes. Biochim. Biophys. Acta 1788, 1731–9 (2009). 3. Vainio, S. et al. Defective insulin receptor activation and altered lipid rafts in Niemann-Pick type C disease hepatocytes. Biochem. J. 391, 465–72 (2005). 4. Morino-Koga, S. et al. Insulin receptor activation through its accumulation in lipid rafts by mild electrical stress. J. Cell. Physiol. 228, 439–46 (2013). 5. Vainio, S. et al. Dynamic association of human insulin receptor with lipid rafts in cells lacking caveolae. EMBO Rep. 3, 95–100 (2002). 6. Czech, M. P. Lipid rafts and insulin action.

Nature 407, 147–8 (2000). 7. Sharma, P. et al. Nanoscale organization of multiple GPI-anchored proteins in living cell membranes. Cell 116, 577–89 (2004). 8. Goswami, D. et al. Nanoclusters of GPI-anchored proteins are formed by cortical actin-driven activity. Cell 135, 1085–97 (2008). 9. Gowrishankar, K. et al. Active remodeling of cortical actin regulates spatiotemporal organization of cell surface molecules. Cell 149, 1353–67 (2012). 10. Raghupathy, R. et al. Transbilayer Lipid Interactions Mediate Nanoclustering of Lipid-Anchored Proteins. Cell 161, 581–594 (2015).

6. Aims and Objective(s) of the current Study

Isolation of primary adipocytes from normal and diabetic human subjects to look at altered plasma membrane organisation, lipid profiles by mass spectrometry to understand the fundamental changes that occur in the membrane composition of diabetic individuals compared to normal subjects • Generation of iPSCs from blood samples obtained from normal and diabetic human subjects which will then be differentiated into adipocytes. This approach is used to do targeted genome editing using CRISPR/Cas9 to knock out genes involved in the building up of membrane domains (vinculin, PGAP2 and PGAP3).

7. Funding Agency:

Wellcome Trust- DBT Margdarshi fellowship/ DST-SERB NPDF

8a. Project start date: 08-Feb-2018 **Project End date:** 08-Feb-2021

8b. Project status: New

9. Summary of research in layman's terms (200 words):

Plasma membrane of the cell is made up of lipid bilayer which is decorated with many proteins known as receptors which recognize specific molecules (ligands) and send messages (signal) to instruct the cell to perform specific function. For efficient functioning of membrane receptors these molecules along with membrane lipids needs to be organized in specialized structures (microdomains- MD) which initiates receptor signaling. My hypothesis is that any alteration in constituents of MD can influence receptor signaling by defective MD assembly. I will test this by studying insulin receptor (IR) signaling as an experimental model. The IR binds to insulin and instructs cells to burn glucose. However, it is not clear whether defective MD assembly due to lipotoxicity leads to obesity associated insulin resistance. I will test this by using mouse models of obesity as well as human stem cells that are lacking specific lipid or protein components important for membrane MD assembly. For this I would require to obtain blood samples from human subjects, reprogram them into iPSCs, perform genome editing and then differentiate them into adipocytes to look at the link between obesity driven change in lipid composition and insulin resistance.

10. Stem cell details:

(i)Type of stem cell:

☐ Embryonic ☐ Fetal ☐ Adult ☑ iPSc

(ii)Model system:

☑ Human ☐ Mouse ☐ Rat ☐ Other

(iii)Mode of study:

☑ in vitro ☐ in vivo

(iv)Transplantation into model organisms:

☐ Yes ☑ No

(v)Human subjects:

☑ Yes ☐ No

11. If human cells/subjects are involved, IEC of human studies approved the proposal? ☑ Yes ☐ No

12. Other committee approvals from NCBS

IAEC approval: ☑ Yes ☐ No ☐ NA

IBSC approval: ☑ Yes ☐ No ☐ NA

IEC approval: ☑ Yes ☐ No ☐ NA

13.Approvals from other collaborating institute (if applicable)"(enclosures may include ethical approvals/Material Transfer Agreement (MTA)/ MoU)"

File attached

File Attachments :

Signature of PI:
Date: 08/02/2018

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ST. JOHN'S MEDICAL COLLEGE & HOSPITAL
INSTITUTIONAL ETHICS COMMITTEE

No : IEC/1/71/2017

16th January 2017

Dr. Arpita Mukhopadhyay
Assistant Professor
Division of Nutrition
St. John's Research Institute
Bangalore – 560 034

IEC Study Ref No. 344 / 2016

Dear Doctor,

Sub : Approval of Research proposal by the I.E.C.

I wish to inform you that your Research Project entitled, **"Calibration of insulin receptor signalling via regulation of local membrane lipid composition"** have been approved by the Institutional Ethics Committee (IEC), SJMCH on **16th January 2017.**

The approval of I.E.C. is valid for a period of ONE YEAR from 16th January 2017 to 15th January 2018.

The study to start only after the submission of CTRI Registration Number.

You must inform the IEC of the following:

1. The Occurrence of Serious Adverse Events (SAE) / AE / Protocol violations and/or Death, during the study period, in the IEC specified format, as per DCGI regulations.
2. Protocol amendment in the IEC specified format
3. (a) Discontinuation (b) Abandonment (c) Completion of this Study, stating the reasons, if the situation of 3(a) or 3(b) is encountered.
4. (a) It is mandatory that a Report for continuing review on the status of the project to be submitted to the Member Secretary in the IEC specified format.
   (b) It is the responsibility of the Principal Investigator to apply for renewal of approval, sufficiently early (**by December 2017)** before the expiry of the existing approval, failing which the existing approval shall lapse.
   (c) On completion of the above Research Project – the Principal Investigator is responsible for submitting a brief summary of the results obtained, to the Member Secretary of the Institutional Ethics Committee at the stipulated time specified by IEC.

With best wishes,

INSTITUTIONAL ETHICS COMMITTEE ★ ST.JOHN'S MEDICAL COLLEGE & HOSPITAL ★ BANGALORE-560 034, INDIA

Rev. Fr. Shaji George Kochuthara
Chairperson

CHAIRPERSON
Institutional Ethics Committee
St. John's Medical College & Hospital
Sarjapur Road,
Bangalore-560 034, India.

CC : The Dean, SJMC / SJRI
The Chief of Medical Services, SJMCH
The HOD for file