

REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1. Name and designation of ICMR- IF : Dr. Aaron Chapla, Lecturer Gr I,
2. Address :
Department of Endocrinology, Diabetes & Metabolism,
Christian Medical College & Hospital, Ida Scudder Road,
Vellore, Tamilnadu, 632004.
3. Frontline area of research in which training/research was carried out : MODY iPSCs & Beta Cell differentiation
4. Name & address of Professor and host institute : Dr. Rohit Kulkarni
Section of Islet Cell and Regenerative Biology,
Joslin Diabetes Center, Harvard Medical School,
One Joslin Place,
Boston, MA, 02215.
5. Duration of fellowship with exact date : October 1st 2019 – 31st September 2020
6. Highlights of work conducted :
 - i) Technique/expertise acquired : hiPSCs generation and characterization
 - ii) Research results, including any papers, prepared/submitted for publication: Digenic mutations in MODY - review article under preparation & also data generated during my training may be considered for publication after validation in the near future.

Highlights of training at Joslin Diabetes Center, Harvard Medical School:

In the first week I had to complete the following mandatory online course to be able to learn and execute mouse model-based experiments:

1. The Animal Research Environment
2. Guide for the Care and Use of Laboratory Animals 8th Edition
3. Technique and Surgical Support and Anesthesia
4. Post-Procedure Care of Mice and Rats in Research: Minimizing Pain and Distress
5. Working with the Laboratory Mouse
6. Working with the Laboratory Rat

Mandatory CITI program online modules completed as a visiting scholar

1. Visiting Scholar / Scientist / Student (ID 31590)
2. CITI Good Clinical Practice Course (ID 31589)
3. Responsible Conduct of Research for Fellows/Visiting Scholars/Students (ID 52777)
4. Conflict of Interest mini-course - Conflict of Interest (ID 60573)
5. Information Privacy Security (IPS) - Research IPS (ID 79310)
6. Information Privacy Security (IPS) - Research Role-Based Training Course (ID 91624)

I have also been able to attend the weekly seminars/data presentations/symposium at Joslin. Some of the interesting sessions are as follows.

Attended Joslin International Symposium "Bioenergetics and its Dysregulation in Diabetes" Held jointly with Banting & Best Diabetes Center and the University of Copenhagen. October 24th-Saturday, October 26th 2019. At Joseph B. Martin Conference Center, Harvard Medical School. During this conference I was able to meet and learn about the ongoing work focused on Beta cell generation and the latest research focusing the pathways in Beta cell development and functioning.

On October 31, 2019, attended the LMA Diabetes and Metabolism Research Lecture Series titled "Precision diabetes medicine and monogenic diabetes" by Prof Pål Rasmus Njølstad, University of Bergen, Haukeland University Hospital, Bergen, Norway.

Rohit's lab and Harvard were a very encouraging environment where I could meet all the fellows in the lab and learn about their work. Some of the important workflows that I have been trained and have gained hands on experience are

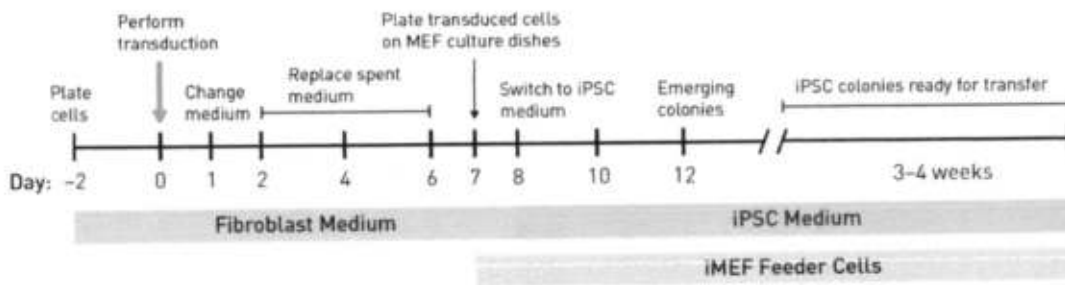
1. iPSCs generation, characterization, maintenance and culture
2. Beta cell differentiation protocol
3. Islet transplantation to mouse kidney capsule
4. Gene editing protocols (MODY 5 mutation)

Along with learning the iPSC based workflow and beta cell differentiation protocols, I was also working on the project entitled

"Identifying gene signatures of beta cells susceptible to develop MODY 5 (HNF1B)".

1. iPSCs generation & characterization

Sendai based reprogramming for iPSC generation

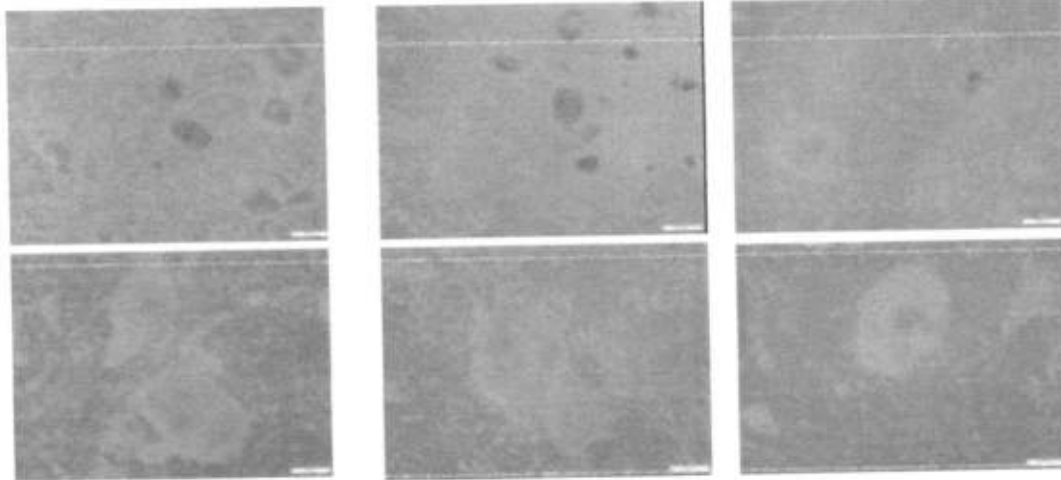


MODY 5 – IPSc colonies at day 18 post transfection

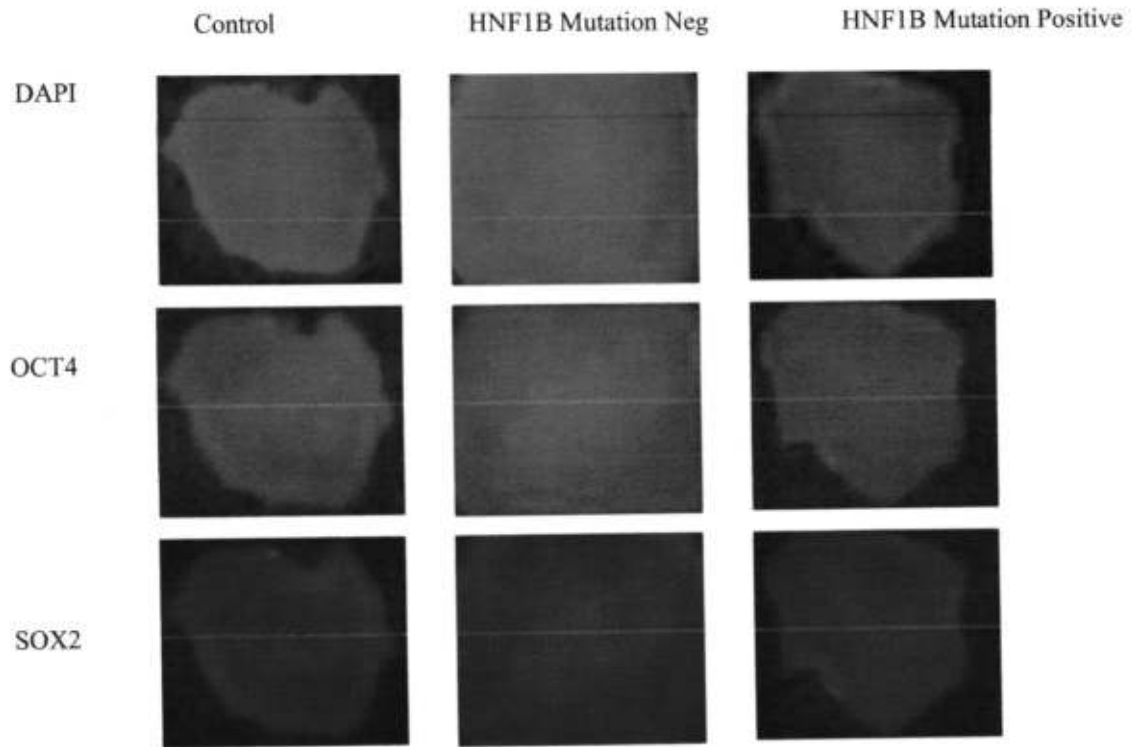
Control

HNF1B Mutation Neg

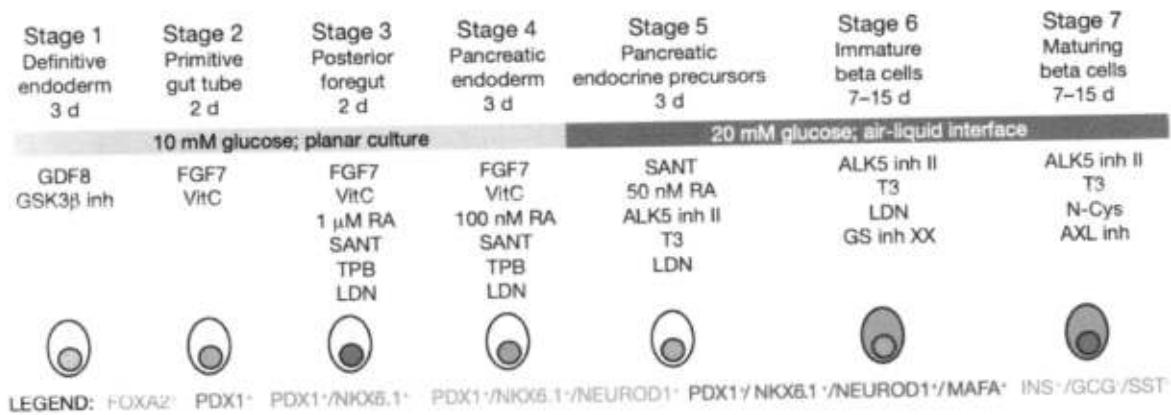
HNF1B Mutation Positive



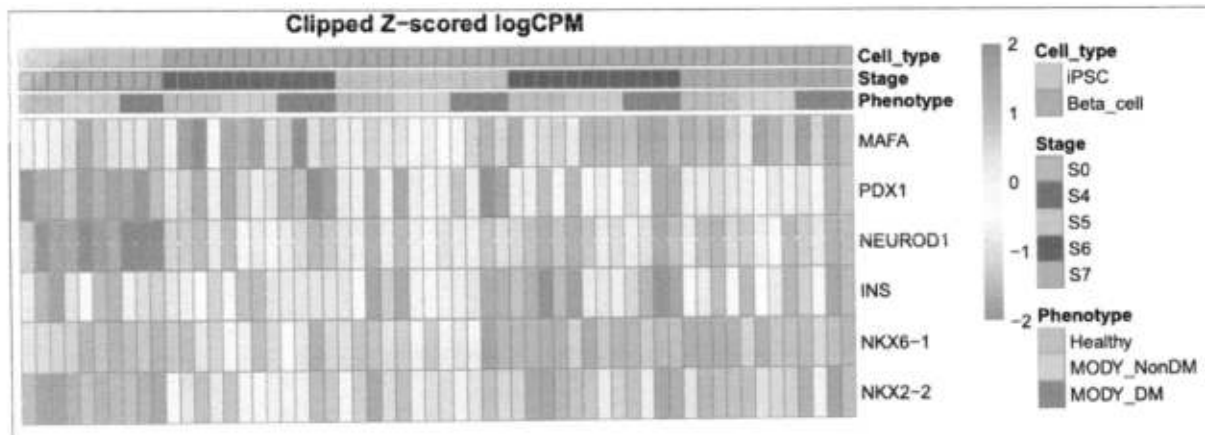
Immunocytochemistry for basic characterizations of iPSC lines : Expression of pluripotency markers, OCT-4A & SOX2



I have learnt the Beta cell differentiation protocol (modified and established in Dr. Rohit's lab) which is based on the published protocol (Rezania et.al., 2014)



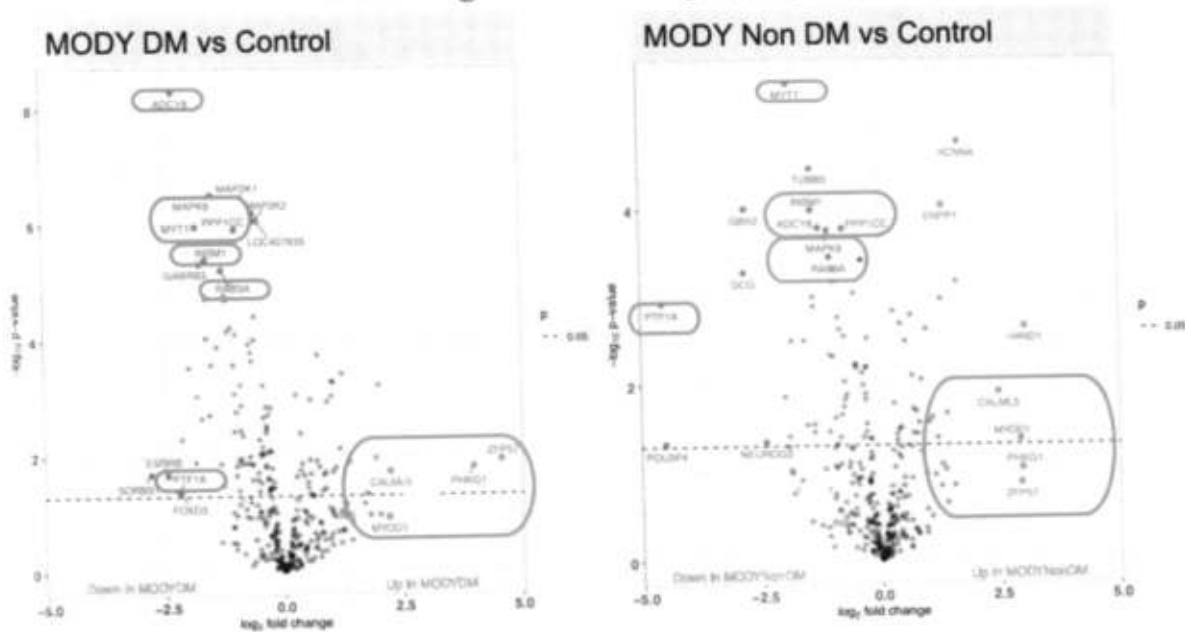
Targeted RNA sequencing data - Gene expression marker for the Beta cell differentiation stage 0 to Stage 7



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We have investigated MODY iPSCs gene signature at stage 0 (iPSC), beta cell differentiation Stages 4 to 7. The samples analyzed include MODY mutation positive subject's (with and without diabetes) vs Healthy controls and also the primary islets from cadaveric donors.

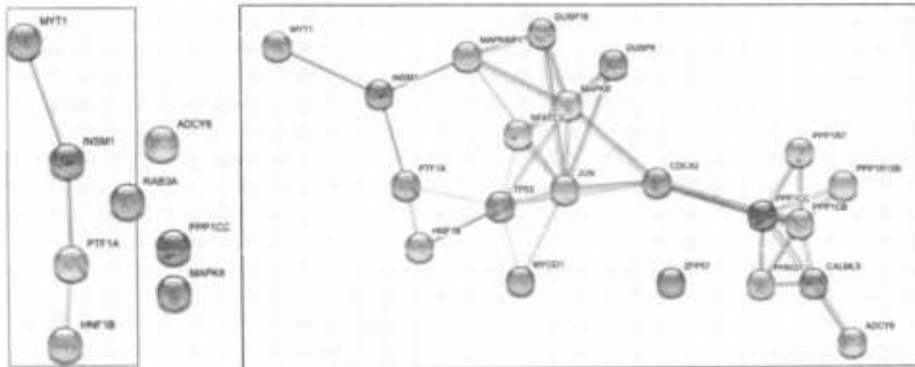
Maturing Beta cell stage (stage 7)



HNFB variant relevant gene expression difference at maturing beta cell stage include down regulation of ADCY8, MAPK8, MYT1, PPP1CC, INSM1, RAB3A, PTF1A and upregulation of CALML5, MYOD1, PHKG1, ZFP57

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Maturing Beta cell (stage 7) – HNF1B mutation specific gene expression



- Therefore, beta cell differentiation stage 7 gene expression may help identify the variants which have a functional impact (Pathogenic) in beta cell development
- Down regulation of PTF1A, INSM1, MYT1 and down stream targets or gene neighbors may help expand the mutant specific gene signature.

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iii) Proposed utilization of the experience in India :

With my training experience at Joslin Diabetes Center, Harvard Medical School, I am working towards getting an adjunct scientist position at Center for Stem Cell Research, Christian Medical College, Vellore. With the allocated space and access to the facility I should be able to establish the MODY iPSC-based investigations and Beta cell differentiation protocols to investigate the role of unique variants identified in young diabetic subjects in India. I believe ICMR DHR IF could prove to be foundational in my research career as I work towards achieving my long-term goal to establish an Islet biology lab in India.

Signature of ICMR-IF

ICMR Sanction No. INDO/FRC/452/ (Y - 23)/201 9-20-/HD

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