University of Eastern Piedmont Amedeo Avogadro

Department of Health Sciences

NOVARA, ITALY

Tutor: **Prof.ssa MARA GIORDANO**

Genetics Laboratory

RANJITH MUNISWAMY

Ph.D in Molecular Medicine

SCREENING GLI2 GENE FOR MUTATION IN CPHD PATIENTS

Annual Report

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1. INTRODUCTION

The past two decades have witnessed an explosion in our understanding of the development of the anterior pituitary gland, and of mechanisms that underlie the diagnosis of combined pituitary hormone deficiency (CPHD). The anterior pituitary is the end-product of a carefully orchestrated pattern of expression of signaling molecules and transcription factors that leads to the development of this complex organ secreting six hormones from five different cell types. These hormones can include growth hormone (GH); follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which both play a role in sexual development and fertility; thyroid-stimulating hormone (TSH); prolactin, which stimulates the production of breast milk; and adrenocorticotropic hormone (ACTH), which influences the production and release of corticosteroids. Energy production in the body and maintains normal blood sugar and blood pressure levels.

1:3 GENETICS OF IGHD/CPHD:

Naturally occurring and transgenic murine models have demonstrated a role for signaling molecules and pituitary transcription factors in the aetiology of IGHD/CPHD. These include the transcription factors HESX1, PROP1, POU1F1, LHX3, LHX4, and SOX3. Depending upon the expression patterns of these molecules, the phenotype may consist of isolated hypopituitarism, or more complex disorders such as septo-optic dysplasia (SOD) and holoprosencephaly. The phenotype and the mode of inheritance can be highly variable. Mutations within the GH-1 and GHRHR genes have shed light on the phenotype and pathogenesis of isolated GHD (IGHD). However to date, mutations have been identified in a modest proportion of patients with IGHD/CPHD and associated syndromes such as SOD.

It is, however, clear that many genes remain to be identified, and characterization of these will further elucidate the pathogenesis of these complex conditions. The recent studies on Sonic Hedgehog (SHH) signaling pathway showed the role zinc finger Gli family proteins in complex disorders of pituitary gland development. The protein secreted in SHH pathway is a crucial entity for the development of ventral forebrain pattern and is required for the separation of primordial eye field and for the distinction of the brain into two halves but if brain failed to separate into two halves leads to the condition called holoprosencephaly [9]. There are three members of the zinc finger Gli family of transcription factors: Gli1, Gli2 and Gli3. Recently mutations within the GLI2 gene have been identified in patients with congenital GH deficiency without other brain defects and most of these patients presented with CPHD [8, 9].

The aim of my project is to search for variations the coding region of the Gli2 gene in a large cohort of familial and sporadic CPHD patients to test the role of this gene in the pathology.

1.4 GLI2 GENE:

The Gli2 gene consists of 13 exons and 2q14 is the cytogenic location and 121,493,440 to 121,750,228 is molecular location on chromosome 2 [10].

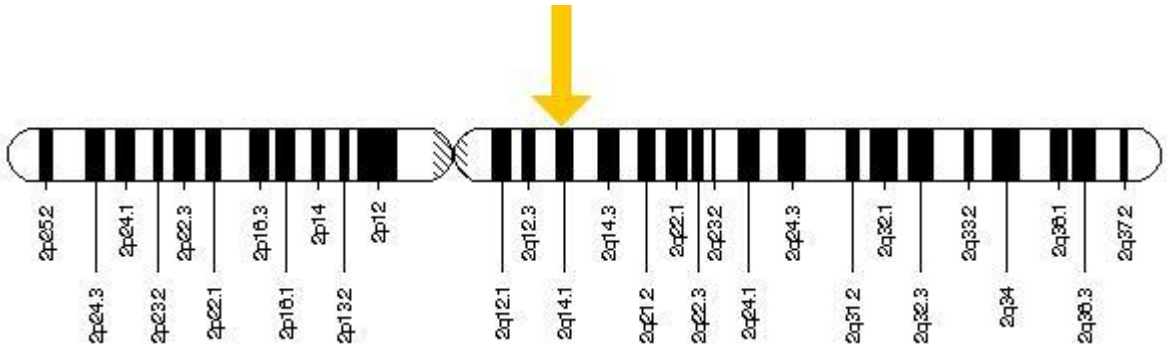

Figure 1- Position of the GLI2 gene on chromosome 2

2. MATERIALS & METHODS:

2:1 DNA EXTRACTION:

Genomic DNA of CPHD patients was extracted from the blood samples by standard salting-out method. The extracted DNA was further amplified by genomiphi kit.

2:2 POLYMERASE CHAIN REACTION & SEQUENCE ANALYSIS:

18 sets of primers were designed to amplify all 13 coding exons of Gli2 gene, using NCBI database (http://www.ncbi.nih.gov/), Primer3 Input (Version 0.4.0, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi/). The primer pair specificity was controlled with the Basic local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Below shown are the primers specifically designed for the project.

Forward Primers	Reverse Primers
5'-TGGGTTTGGGCTCAGTGT-3'	5'- CCTCTTCGCCCTCCATAAAC-3'
5'-TGGCTGCTCTTGCTATGAAA-3'	5'- GCAGGAGATGTGGCTGAGG-3'
5'-CATGTTGGTTTTGGGGTCTT-3'	5'- GACCAAGGCTGAGGAGTTGA-3'
5'-TGTGCATTTCTCTCTGCCTTT-3'	5'-CCTTGTCCCCAAAAGAAACA-3'
5'-CCTTGCAGGCTCTTCCTATC-3'	5'-TCTTTCTCCTCGGGTCAAAA-3'
5'-TGGGCAAGGTTCTCTCTGTC-3'	5'-CTTAGCATGAGCTGGCAGTG-3'
5'-TGTGCGGAGAGATCCTAGAG-3'	5'-TTCACCACCAAGGGTACAGC-3'

5’- TTCCCCACAGCACTTCGAT-3’	5’- TCCAGCCCCTTCTGTCTAGT-3’
5’- GACAGCAGGGGGTGGTCT-3’	5’-CCACCTCCAAACATGATCC-3’
5’-GGTTGGAGCAGAGCAGAGAA-3’	5’-GGCACCTGGCTATCTACTGG-3’
5’- CGTGGGTAGCTTCAGGAGAA -3’	5’-GATATCGCTGTGCCCCTAGA-3’
5’-CTTCCTTCCCTCACCCTCAG-3’	5’-ACTGGGCTGGTAAGATCCTG-3’
5’-GTGTTGCAAGCCCTCTTCTC-3’	5’- AGTGGCTGCCGCGTACTT-3’
5’-CTACTTCTCCAGCCGCCG-3’	5’-ACGTTCTCGCTGATGCTAGG-3’
5’-CTTCCACAGCACCCACAAC-3’	5’-CCTTGCGGACTGTAGCCC-3’
5’-GCAGTGGAATGAGGTGAGCT-3’	5’-GATGGCTCTGCTGTGGGTAG-3’
5’-CCCTCAGCAGACAGAAGTGG-3’	5’-GTACATGTGGATCTGGCCGT-3’
5’- CAGTCAGGAAACAGCAGAGG-3’	5’-GGAAAAAGACAAGACAGCTGGA-3’

Table 1: Showing list of Primers designed for amplifying specific coding region in Gli2 gene.

The PCR reaction was carried out with the GoTaq Flexi DNA polymerase (Promega) in a 15µl reaction volume, with touchdown PCR protocol from 65^0 C to 55^0 C annealing temperatures. The initial denaturation at 94° C for 5 min, 20 cycles consisting of 30s denaturation at 94° C, 30s annealing at higher temperature 65° C and 30s extension at 72° C, followed by second cycle consisting 25 cycles of denaturation at 94^0 C for 30 sec, annealing at lower temperature 55^0 C and extension at 72^0 C, followed by a final extension

at 72° C for 7 minutes and cooled to 4^0 C. The amplified PRC products were run on a 2% Agarose gel to confirm the PRC amplification.

The PCR products were purified using Affymerix Exo/SAP-IT enzymatic PCR clean up system (37° C 15 min and 80° C 15 min) and directly sequenced with Big Dye Terminator kit (Applied Biosystems) and automatic sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The Sequencing Reaction was carried out in a Thermo cycler with the following conditions: 3 minutes at 96° C 25cycles consisting of 20 seconds at 96° C 5 seconds at 50° C and 4 minutes at 60° C and finally cooled to 4° C. The sequence reaction products were purified by precipitation using ethanol and 3M Sodium Acetate (pH 5.2) and resuspended in 12µl formamide.

3. RESULT:

The amplification and sequencing of the Gli2 gene have been performed for the first 6 exons. To date no mutation has been identified in these first exons. The mutational analysis is still in progress. We are planning to increase the number of patients for screening from 95 to 285 patients, also including IGHD patients.

4. REFERENCES:

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5. CONFERENCE:

- *9th SIBBM* - Frontiers in Molecular Biology Revisiting the Central Dogma: Emerging New Concepts in Replication, Transcription, and Translation - **Pavia, 5-7 June 2013.**

6. ABSTRACT:

- INCIDENCE OF *SHOX1* MUTATIONS IN PATIENTS WITH SHORT STATURE AND VARIABLE GROWTH RETARDATION RATE.

Ileana Fusco[1], Simona Mellone[1], Deepak Babu[1], Ranjith Muniswamy[1], Flavia Prodam[2], Simonetta Bellone[2], Antonella Petri[2], Gianni Bona[2] and Mara Giordano[1].1-Laboratorio di Genetica Umana, Dipartimento di Scienze Mediche, Università del Piemonte Orientale, Novara 2-Università del Piemonte Orientale, Dipartimento di Scienze Mediche, Unità di Pediatria, Novara.

Annual Meeting, Società Italiana di Genetica Umana, September 25-28, 2013

7. POSTER:

- *DAL DNA ALLE MALATTIE* – Poster from Genetics Labarotory on **September 27, 2013** for *Ph.D Day* in University of Eastern Piedmont Amedeo Avogadro, Alessandria, Italy.

8. SEMINARS:

- Cardiovascular risk in nephrology, dialysis and transplantation: combine the results of the research to the needs of clinical practice - Prof. John Strippoli University of Sydney School of Public Health, Australia, Mario Negri Sud Consortium, University of Bari, Italy.
- Stem/progenitor cell transplantation in the rat: A powerful tool to study tissue replacement in the normal and diseased liver - Michael Oertel, PhD Assistant Professor, School of Medicine Dept. of Pathology, University of Pittsburgh (USA).
- Health properties of natural phytochemicals/Neurochemical properties of rhinacanthus nasutus extracts/ Protection against UVB damage in skin cells by Thai herbs extracts - Prof. Dr TEWIN TENCOMNAO Department of Clinical Chemistry Faculty of Allied Health Sciences Chulalongkorn University, bangkok (Thailand).
- Heparan Sulfate: A versatile target for the development of new drugs - Giancarlo Ghiselli, President Glyconova Srl, Startup Bioindustry Park "Silvano Fumero", Colleretto Giacosa, Turin.
- Cytotoxic potential of plasmacytoid dendritic cells in autoimmune diseases - Prof. Silvano SOZZANI, Department of Molecular and Translational Medicine, University of Brescia.
- Skin cancer in vivo models, what they have and can tell us – Dr. Girish Patel, Department of Dermatology and Wound Healing School of Medicine, Cardiff University, United Kingdom.
- Cancer in the post-transplant: emerging issues and new opportunities to care - Prof. Emanuela Vaccher, Medical Oncology A, Oncological Referral Center, Aviano IRCCS, San Raffaele del Monte Tabor Foundation, Milan.
- Molluscum contagiosum virus: Strategies for survival in the human epidermis - Speaker: Dr. Joachim Bugert, Cardiff University School of Medicine.
- SINEUPs: a new functional class of antisense non-coding RNAs that activate translation " Speaker: Stephen GUSTINCICH SISSA Trieste.
- Red blood cells as carriers for magnetically targeted delivery of drugs" Speaker - Dr. Hans Bäumler, Head of the Research Department, Institute of Transfusion Medicine,

Berlin -Brandenburg Center for Regenerative Therapies Charité – Universitätsmedizin, Berlin.

- Interleukin -33: a novel player in chronic intestinal inflammation. Dr. Luca Pastorelli Department of Biomedical Sciences for Health, IRCCS Policlinico San Donato San Raffaele del Monte Tabor Foundation, Milan.
- Autophagy and human disease; model Cystic Fibrosis. Prof. Luigi Maiuri, University of Foggia, European Institute for Research in Cystic Fibrosis, Division of Genetics and Cell Biology, IRCCS S. Raffaele Milan.
- Experimental and Clinical Applications of polychromatic cytometry and multi-purpose. Prof. Andrea Cossarizza, University of Modena and Reggio Emilia.
- Carbapenemases: a last frontier for beta-lactam antibiotics? Prof. Giuseppe Cornaglia, Department of Pathology and Diagnostics, University of Verona.
- Glioblastoma stem cell biology and its implications in cancer therapy. Speaker - Dr. Giuliana Pelicci, Director of 'Unit "Biology and signal transduction of normal neural stem cells and cancer cells" Department of Experimental Oncology, European Institute of Oncology in Milan.
- MRNA & Genome Editing. Speaker - Dr. Angelo Lombardo San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET) Milan.
- CAV1 protein in Skin Cancer Pathogenesis. Speaker-Dr. Franco Capozza, Thomas Jefferson University in, Philadelphia, PA, USA.
- Molecular interactions of histone modification and de novo DNA methylation. Speaker - Dr. Kyung-Min Noh, PhD, Rockefeller University, New York, NY (USA).
- Medical Implications of oral pathologies of infectious-inflammatory character Speaker- Prof. Roberto Abundo.
- Long non-coding RNAs and atypical protein-coding genes as new players in the regulation of neuronal functions.
- Alumina/Zirconia Composites for hip joint applications: State of the Art, Market & Future Trends. Speaker - Dr. Alan Alexander Cardinals, PhD, Manager of Scientific Affairs, Medical Products Division, CERAMTEC GmbH.

- Preventing cancer with vaccines. Speaker - Dr. Lacopo Baussano, University of Eastern Piedmont , IARC Lyon
- Mechanisms of chronic inflammation and immunoregulation in infections and tumors. Speaker - Vincenzo Barnaba, La Sapienza University of Rome
- Lactoferrin and its many functions. Speaker - Prof. Piera VALENTI. University of Rome "La Sapienza".
- Helps: biomimetic polypeptides for biomedical applications. Speaker - Dr. Antonella Flag, Department of Life Sciences, University of Trieste.
- The Nrf2 transcription factor in disease, aging and stem cell function. Speaker - Prof. Dirk Bohmann, Professor of Genetics, Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY (USA).
- LR2 and CD44 in the modulation of experimental MS: direct and indirect effects on autoreactive T cells. Speaker - Francesco Ria Catholic University of the Sacred Heart, Rome.
- Searching for novel genes and micro RNAs inducing myocardial protection and regeneration. Speaker - Mauro Giacca, MD, PhD Professor Molecular Biology, University of Trieste, Director of International Centre for Genetic Engineering and Biotechnology, Trieste.
- Development of a new genetic model for outcome prediction in chronic lymphocytic leukemia. Speaker - Dr. Lorenzo De Paoli, PhD in Clinical and Experimental Medicine, XXV CYCLE
- Developing Vaccines against Polyomaviruses". Speaker - Prof. Christopher Buck, Professor in Immunology, Laboratory of Cellular Oncology, Head of the Tumor Virus Molecular Biology Section, National Cancer Institute – Bethesda, Dunn School of Pathology, Oxford University.
- Plasma membrane expression of GLUT1 in Thyroid Carcinoma: characterization of molecular pathways and clinical implications. Speaker - Dr. Federica Morani PhD in Clinical and Experimental Medicine, XXV CYCLE.

- Alcohol, Cigarette Smoking and Multiple Sclerosis: the results from a case control study. Speaker - Dr. Andrej Ivashinka, PhD in Clinical and Experimental Medicine, XXV CYCLE.
- The UHRF1 protein promotes epigenetic cross-talks and cancer progression is involved in. Speaker - Prof. Marc Bonapace Dep. of Theoretical and Applied Sciences Division of Biomedical Sciences, University of Insubria.
- Biocompatible supports for tissue engineering. Speaker - Dr. Luca Fusaro, PhD in Clinical and Experimental Medicine, XXV CYCLE.
- T-cell antigen receptor: a journey through early and late signaling. Speaker - Prof. Oreste ACUTE, Professor in Immunology, Dunn School of Pathology, Oxford University.
- Whole of DNA sensor IFI16 in the development of autoimmunity: clinical and molecular studies. Speaker - Valeria Caneparo, Ph.D. in Clinical and Experimental Medicine, XXV CYCLE.

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