GALACTOSEMIAS AND OTHER INBORN ERRORS OF METABOLISM

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Background

Inborn errors of metabolism are a group of rare genetic diseases characterized by failure of the metabolic pathways involved in either the catabolism or storage of carbohydrates, fatty acids, and proteins. In this way, metabolites accumulate and can be toxic or interfere with normal function, or the synthesis of essential compounds can be reduced. These disorders are usually caused by mutations in the genes coding for those enzymes that work in the metabolism of the nutrients, leading to the impairment of the enzymatic activity, or to the destabilization of these proteins.
In this research line, we use several computational approaches with different aims. First of all, we would like to identify the effects of the mutations on the proteins' structure and function, in order to establish a clear-cut correlation between the genotype and the phenotype of the patients, able to anticipate the long-term complications of a particular mutation and to prevent the more severe effects. Secondly, we would like to identify possible therapies for some diseases, in particular by applying the concept of "pharmacological chaperones".
Inborn errors of galactose metabolisms, collectively known as "galactosemias" are used as model diseases to apply this kind of approaches, which can be extended to other enzymes and proteins involved in other inherited metabolic diseases.

Main achievements

The responsible of this research line has a long-standing activity in the field of computational biology approaches applied to galactosemia. For the first aims, the responsible, together with other researchers of the Institute of Food Science,

CNR, Avellino (see "External collaboration" section) has developed a Web-accessible database for the storage and interrogation of the information about the impact of mutations on structures and functions of the enzymes associated to the different forms of Galactosemia. The database, accessible at the Web site http://www.protein-variants.eu, at present collects data about 360 mutations affecting the four enzymes of the galactose metabolism, i.e. galactose mutarotase (GALM), galactokinase (GALK1), galactos-1-phosphate uridylyltransferase (GALT) and UPD-galactose epimerase (GALE), together with data about the wild type proteins and other information useful for clinicians and patients. The database is organized with multiple levels of complexity, in order to facilitate the retrieval of correct information also by non-experts. This resource can also be accessed programmatically and is part of ELIXIR, the European distributed infrastructure to support life science research (https://elixir-europe.org/). It is continuously supported and updated, and there are plans for a further expansion (see "Future perspective" section).

Future perspectives

Our future activities are focused on several different goals. First of all, we would like to expand the current database and Web server in order to host data also for other enzymes involved in different inborn errors of metabolism. Indeed, our conceptual approach can be easily translated to other protein targets, and this would results in an increased comprehension of the phenomena that lead to enzymatic inactivation in this kind of diseases, with benefits on the diagnosis and the search for therapeutic approaches. Secondly, the characterization of the effects of mutations would be more efficient and exhaustive with the development of automated methods for the modelling and analysis of the mutants of the proteins, and we are intended to pursue this scope, given also the opportunity of fundings obtained by the participation of the responsible to a PRIN national project. Finally, we are working on the identification of possible pharmaceutical chaperones able to stabilize and rescue the enzymatic activity of GALT enzyme and in particular of its more different mutation, p.Q188R, in order to counteract the negative effects on protein activity and stability caused by this mutation.

Publications

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10. *Marabotti A§ and Facchiano AM. Homology modeling studies on human galactose-1-phosphate uridylyltransferase and on its galactosemia-related mutant Q188R provide an explanation of molecular effects of the mutation on homo- and heterodimers. J Med Chem. 2005; 48,773-779.*

External collaborations

- *Facchiano A, Institute of Food Science, CNR, Avellino, Italy*
- *d'Acierno A, Institute of Food Science, CNR, Avellino, Italy*

••• Laboratory •••

Cell cultures/Intestinal cellular models
Cell culture facilities are indispensable for basic research and a wide range of clinical in vitro studies. This facility has a dedicated laboratory of cell culture and intestinal cellular models. Intestinal organoids, long term cultured crypts from human duodenum, rectum and from mouse intestine, have been established, implementing 3D and 2D cell cultures. Three-dimensional cultures include multicellular cultures, scaffold cultures and spheroid cultures.

Molecular biology
The Molecular Biology Service is a facility providing research support for a range of molecular biology techniques. DNA and mRNA from intestine and other cellular compartment including blood cells are collected from patients and control subjects to create a large bio-bank. Moreover our facility provide a training environment in which our researchers can acquire and use molecular biology techniques both for basic research as well as for diagnostic tests.

Microscopy
The confocal microscopy facility provides microscopes to visualize a variety of samples, from monolayers and small organisms, such as 2D, 3D and 4D cultures. These technologies provide us with the highest light microscope resolution obtainable, giving the scientist a clearer picture of cellular and subcellular structure and function. Single or multiple labeled specimens can be analysed using magnifications from 10X to 100X with a temporal resolution up to 400 fps. Our laboratory has experience in imaging of mammalian cells, tissues sections of live specimens. The laboratory also maintains several workstations with software packages for image processing, analysis, and 3D image reconstruction.

Biochemistry
Biochemistry of the proteins is a main tool for the understanding of biological process through the assessment of protein quantity and interactions. The facility offers both the technology and the technical support for western blot analysis, immunoprecipitation and ELISA test.

Mucosal immunology
Lymphoid cells are obtained from biopsy specimens and separated from the different compartments. T cell lines and clones are routinely established. Flow cytometry is used for cell analysis and isolation.

Organ cultures

The main goal of this facility is to set up organ culture of intestinal biopsies. The facility is also responsible of a wide bio-bank of cells (fibroblast), intestinal tissues, serum, DNA and RNA from patients with celiac disease at all stages of disease (potential, active and remission phases), as well as from subjects with diagnosis of food allergy and inflammatory bowel diseases.

Pathology and lab diagnostic test

A pathology lab is associated to the Center. Routine histology and immunohistochemical/ immunofluorescent studies are currently applied to the diagnosis of food allergy and gluten sensitive enteropathy. Count of gamma delta lymphocytes and assessment of the presence of intestinal deposits of IgA anti-tissue transglutaminase2 are currently performed. For diagnostic purposes, organ culture of small intestinal biopsies is implemented in special situations with immunohistochemical analysis and measurement by ELISA test of CD associated antibodies in the supernatant.

Several non-invasive diagnostic tools such as intestinal permeability test and inflammatory markers' measurement (determination of fecal levels of calprotectin and/or eosinophilic cationic protein) are routinely used in the diagnostic approach and in the follow up of children with food-induced diseases. In addition, skin prick test, specific serum immunoglobulin E measurement, and atopy patch test are currently used as part of the diagnostic approach to children with suspected food allergy. Sugar breath test are routinely performed for the diagnosis of sugar intolerance, and more recently new diagnostic approach based on molecular analysis of the specific genes, has been developed for selected food-induced diseases (e.g.: sucrose-isomaltase deficiency, glucose-galactose malabsorption, fructose malabsorption).

••• Clinical •••

ELFID is associated to the Department of Mother and Child Health of the University Hospital "Federico II". For the implementation of research activities special connections have been established with the following Sections.

The Celiac Disease Center, serving the whole Campania Region, offers the possibility of diagnosis of the disease, also in unclear cases with the help of advanced histological techniques and genetic investigations. Celiac children are also followed up on a gluten free diet with scheduled visits by paediatrician and nutritionist to monitor growth, compliance to the diet and onset of any clinical and nutritional complication. Special interest is paid to the children with potential celiac disease. Cohort of at risk children are followed-up in view of possible prevention programs.

The GI Endoscopy and Motility Suite provides pediatric investigations for diseases of the GI tract, including food-induced diseases. The Unit is committed to training, development and education of its staff and offers opportunities for research.

GI Endoscopy. Among diagnostic procedures the Unit offers upper and lower GI endoscopy and wireless capsule endoscopy. GI endoscopies are done under anesthesia or under deep sedation in the operatory room and in a renewed endoscopy suite, respectively. The main indication for upper GI endoscopy for food- induced diseases are celiac disease and food allergy, in particular eosinophilic esophagitis and eosinophilic colits.

pH studies and manometric studies. The unit offers multichannel intraluminal impedance that allows for detection of flow throughout the esophagus. It is, therefore, able to detect all gastroesophageal reflux (GER), whether acid, weakly acid, or weakly alkaline. It not only detects all GER but also enables us to more accurately reveal associations between GER and symptoms. It finds application in the diagnostic approach of children with suspected food induced diseases (e.g. eosinophilic esophagitis, secondary gastroesophageal reflux disease). Esophageal, antroduodenal and anorectal motility studies can be performer by high-resolution pressure topography (HREPT). HREPT has several advantages compared with conventional manometry, the technology that it was designed to replace. Compared with conventional manometry, HREPT has improved sensitivity for detecting achalasia, largely due to the objectivity and accuracy with which it identifies impaired esophago-gastric junction relation. The main indication for GI manometric studies in food-induced diseases is food allergy-related chronic constipation.

Food Allergy Comprehensive Education, Treatment and Support Program is designed to treat all aspects of food allergies, including medical, dietary, social and psychological concerns by a multidisciplinary team composed by pediatricians, allergists, dieticians, nutritionists, social workers, nurses, psychologists, neuropsychiatrists, and allergy biotechnicians. The Program has been accredited as the Reference Center for Pediatric Allergy by the Italian Society of Allergy and Pediatric Immunology (SIAIP) (http://centri.siaip.it/).

Main activities of the Program are:

- Integrated clinical, instrumental and laboratory management for pediatric allergic diseases (food, drug, and environmental allergies).
- Personalized nutritional counseling for effective dietary programs for the prevention and treatment of food allergies with a full assessment of the nutritional status.
- Active diet-therapy plans based on pro-/post-biotics, baked foods and functional foods to speed up the acquisition of immune tolerance.
- Allergen specific immunotherapy with biological drugs.

Integrated Diagnostic Work-up Procedures:

The diagnostic work-up consists in a broad range of in vivo and in vitro tests, including:

- Skin prick testing (SPT) for the first line approach in IgE-mediated diseases.
- Atopy patch testing (APT) for the evaluation of patients with delayed type hypersensitivity reactions.
- Total and specific IgE assay for the evaluation of total and specific serum IgE against food allergens by enzymatic immunoassay.
- Molecular allergy diagnostic tests with component-resolved diagnostics (CRD) to differentiate primary, species-specific from secondary, cross-reactive sensitizations to single food allergens.
- Basophil activation test (BAT) using flow cytometry for the assessment of basophil activation after exposure to a specific food antigen to investigate IgE-mediated reaction, to limit the risk of oral food challenge, and to monitor immunotherapy.
- Spirometry for the evaluation of subjects with food-induced-asthma.
- Provocation tests for inducible urticaria are performed using physical stimuli (friction, water, cold, heat, weight) for the differential diagnosis with food-induced conditions and for the diagnosis of symptomatic dermographism, delayed pressure urticaria, acquogenic, heat and cold-induced urticaria.
- Oral food challenge (OFC) the gold standard procedure to obtain an accurate diagnosis of food allergy and consists of a gradual feeding of the tested food under close observation.
- Allergen Specific Immunotherapy is available for desensitization protocols in IgE-mediated food allergy patients.

Clinical Nutrition Unit

A personalized nutritional counseling, provided by a team of experienced dieticians and nutritionists, drive a modern dietary program with the aim to avoid allergic reactions, help all phases of the diagnostic work-up (including the diagnostic elimination diet and oral food challenge), ensure normal body growth and development, and stimulate immune tolerance.

An advanced nutritional evaluation is provided to the patients through the use of:

- Plicometry, a non-invasive method to measure body adiposity.
- Indirect calorimetry, a non-invasive method to investigate basal metabolism.
- Bioelectrical Impedance Analysis (BIA), for the estimation of body composition, in particular body fat and muscle mass.
- Dual X-ray Absorptiometry (DEXA), for the evaluation of bone growth and of body composition (bone mass, fat mass and muscle mass).
- BOD POD (Gold Standard Body Composition Tracking System), a fast and non-invasive tool which is considered the Gold Standard for the assessment of body composition, basal metabolism and energy consumption.

Liver Unit

The Liver Unit strictly cooperates with the pathologist to offer histological definition of several types of hepatobiliary disease. Liver biopsy is performed with ultrasonography assistance. Particular interest is paid to obesity related diseases and metabolic and autoimmune liver diseases.

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