"21-Maps Projects": A route to the discovery of a cure for trisomy 21 (Down Syndrome)

Laboratory of Genomics

Experimental, Diagnostic and Specialty Medicine - DIMES
Activity of Istology, Embryology and Applied Biology
University of Bologna

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"We will beat this disease. It's inconceivable that we won't.
It will take much less intellectual effort than sending a man to the Moon.
If I find out how to cure trisomy 21, then that would clear the way for curing all the other diseases that have a genetic origin."

Jérôme Lejeune (on the therapy for Trisomy 21)

21-Maps Projects: A route to the discovery of a cure for trisomy 21 (Down Syndrome)

1. Indroduction

Down syndrome (DS) is a genetic condition that occurs as a result of an extra chromosome (chromosome 21).

DS is the most frequent human chromosomal disorder, with a frequency of 1 in ~400 conceptions and 1 in ~700 births.

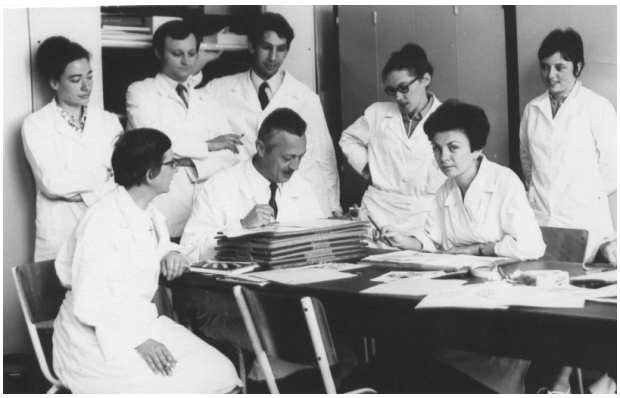
This chromosomal disorder causes a “syndrome”, a set of physical and mental "traits" originally described in 1866 by John Langdon Down (for whom the syndrome is now named).

There can be great variability in the severity of symptoms. Main symptoms include distinct facial and physical features among which are almond shaped eyes, flat nasal bridge and short stature.

Cognitive impairment is present to some degree of severity in all affected individuals and involves symbolic thought.

In 1959 Prof. Jérôme Lejeune demonstrated that the syndrome results from a condition called trisomy 21, a genetic mutation leading to the presence of three copies of human chromosome 21 (Hsa21) in the cells of affected individuals instead of the normal two (Lejeune et al. 1959).

The research field on trisomy 21 was brought to the University of Bologna by Prof. Maria Zannotti, now retired, who, in the late '60s, was a pupil of Prof. Lejeune in Paris. The research group she created, now under the supervision of Prof. Pierluigi Strippoli, is continuing the Down Syndrome research program using the new tools provided by the completion of the "Human Genome Project".



Parigi, 1969 - La Prof.ssa Maria Zannotti (la seconda da destra) in riunione con il gruppo del Prof. Jérôme Lejeune (seduto al centro).

The study is carried out in collaboration with Prof. Guido Cocchi, Neonatology Unit at S. Orsola-Malpighi Hospital, Bologna and is approved by the competent Ethics Committee of Sant'Orsola-Malpighi Hospital.

2. Results to date (May 2016)

2.1 Creation of transcriptional maps for human brain, hippocampus and blood cells to study expression of human genes in detail, in particular of chromosome 21 (see the following publications):

http://www.ncbi.nlm.nih.gov/pubmed/25185649
http://www.ncbi.nlm.nih.gov/pubmed/26108741
http://www.ncbi.nlm.nih.gov/pubmed/25476127

2.2 Reanalysis of all reported partial trisomy 21 cases aimed to identify, on the human chromosome 21, the critical region responsible for the intellectual disability in Down syndrome. A manuscript presenting the results of this research was published in April 2016 in "Human Molecular Genetics":

http://www.ncbi.nlm.nih.gov/pubmed/27106104/

2.3 To date, a bank of biological materials is available in our laboratory: blood samples; DNA and RNA extracted from blood samples; plasma and urine collected from the 75 enrolled individuals with DS and 32 control subjects. We have the first preliminary results of the metabolome analysis performed through NMR (nuclear magnetic resonance) in urine samples.

3. New goals for 2016-2017

The purpose of this project is to identify DS-specific molecular markers as potential therapeutic targets.
The identification of the "Down syndrome critical region" was a fundamental breakthrough for our research and on the basis of these results we have defined new goals:

3.1 Characterization of Highly Restricted Down Syndrome Critical Region (HR-DSCR). Characterization of new genes located on the critical region for DS intellectual disability in order to identify new genes as essential for DS manifestation.

3.2 Metabolomic profiling in biological samples from subjects with DS.
We will proceed to collect blood, plasma and urine in order to expand the biological bank . Starting from plasma and urine samples, we are going to explore the metabolome profile to link specific alterations to genetic determinants on Hsa21, in particular to HR-DSCR. The analysis of profiles will focus on metabolites related to one-carbon/folate pathways in order to identify possible points of therapeutic intervention.

3.3 In vitro study using primary cell cultures. The cells (trisomic and disomic fibroblasts) will be used to test the effects of various forms of folate in rescuing the antiproliferative effects of the antifolate methotrexate (MTX), to which DS cells are hypersensitive. The identification of a form specifically bypassing the block in the trisomic cells in comparison with diploid cells may lead to a clinical trial using the appropriate form of folate.

"The whole difficulty of the research is how to discover the discordant musician, because the orchestra of life has about fifty thousand musicians." (Jérôme Lejeune)

Methods are described in detail here:

http://www.spp-j.com/spp/1-1/spp.2013.12R0005/

5. Chief Scientist Curriculum - Prof. Pierluigi Strippoli

The chief scientist of the project is Prof. Pierluigi Strippoli, Associate Professor of Applied Biology at the School of Medicine and Surgery of Bologna University.

https://www.unibo.it/sitoweb/pierluigi.strippoli/cv-en

6. Research Group

The operative unit will perform the research project in the Laboratories of Genomics and Post-Genomics, Department of Experimental, Diagnostic and Specialty Medicine (Activities of Histology, Embryology and Applied Biology), University of Bologna.
In particular, the project will be carried out by:

First and Last Names	Degree	Role
Pierluigi Strippoli	Medicine and Surgery	Associate Professor
Lorenza Vitale	Medicine and Surgery	Researcher
Maria Chiara Pelleri	Biotechnology	Post-doc
Allison Piovesan	Biotechnology	Post-doc
Maria Caracausi	Biotechnology	Post-doc
Francesca Antonaros	Biotechnology	Post-Degree Fellow
Gabriella Mattei		Laboratory technician

We will take advantage of the collaboration of Prof. Maria Zannotti, formerly Associate Professor of Applied Biology at the University of Bologna and now retired.

6.1 Recent National and International Collaborators

Prof .Guido Cocchi
Neonatology Unit, St. Orsola-Malpighi Polyclinic, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy

Dr. Chiara Locatelli
Neonatology Unit, St. Orsola-Malpighi Polyclinic, Bologna, Italy

Dr. Maria Chiara Mimmi
Department of Medical and Biological Sciences, University of Udine, Udine, Italy

Dr. Anna Concetta Berardi
Research Laboratory "Stem Cells", U.O.C. Immunohematology-Transfusion Medicine and Laboratory of Hematology, Santo Spirito's Hospital, Pescara, Italy

Dr. Doris Ricotta
Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

Prof. Donatella Barisani
Department of Health Sciences, University of Milano Bicocca, Monza, Milan, Italy

Prof. Mark Basik
Laboratory of Cancer Genomics in the Department of Oncology and Surgery at the Lady Davis Institute of McGill University in Montreal, Canada

Dr. Maria Chiara Monaco
Laboratory of Molecular Medicine and Neuroscience, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA

Prof. Marco Seri
Medical Genetics Unit, St. Orsola-Malpighi Polyclinic, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy

7. Funding

A cost of 150.000,00 Euro/year had been estimated for the first part of the Project (Summer 2013 – Summer 2016), of which 75.000,00 Euro for fellowships.

We actually raised a mean of 73.614,5 Euro/year, about a half of the amount necessary for the initial Project. However, based on novel results obtained during the study, we chose to not carry out the "Next Generation Sequencing", which we have estimated would require 60% of the costs for the experimental plan. We have therefore been able to achieve our essential objectives.

To date, the funds raised have come from free donations (85%) and institutional sources based on competitive applications. 71% of total funds were necessary for the fellowhips.

Funding sources have been ackowledged in the "Acknowledgements" section of the published articles.

For the period **Summer 2016 - Summer 2019**, based on the new objectives indicated above, we estimate a cost of **105.000 Euro/year** (**70.000** Euro/anno for fellowships and **35.000** Euro/year for the expenses of the Laboratory).

The following division between several items is only indicative (changeable in detail depending on the experimental requirements that arise during the study):

Biologia Molecolare/ SpeseAccessorie	
Plastiche e reagenti di laboratorio	2.500,00
Puntali, provette	2.500,00
Reagenti per PCR, gel e purificazione del DNA	3.300,00
Sequenziamento del DNA	1.200,00
Reagenti per clonaggio in vivo	2.000,00
RNA e cDNA	3.500,00
Reagenti per Northern-blot	2.500,00
Reagenti per "Real-Time" PCR	2.500,00
Reagenti per saggi funzionali	5.200,00
Hardware/software per analisi dati	2.800,00
Spese di pubblicazione dei risultati	4.000,00
Spese per missioni/collaborazioni [Partecipazioni a congressi e riunioni di lavoro, organizzazione di seminari]	3.000,00
Totale per un anno	**35.000,00**

Use of donations

All donations to the Laboratory will be used for the following purposes:

1. Funding fellowships for young researchers (Ph.D. students and Postdocs) working on the project

2. Purchasing materials, reagents and services needed for the experimental work.

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