"Genome 21 Project":
A route to the discovery of a cure for trisomy 21 (Down Syndrome)

Laboratory of Genomics

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

Last updated on March 14, 2018

"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 genetic disorder, with a frequency of 1 in ~400 conceptions and 1 in ~700 births.

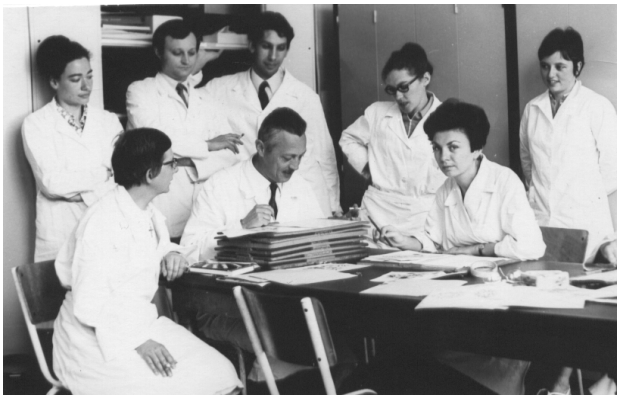
This chromosomal disorder causes a “syndrome”, a set of physical and mental "traits" originally described in 1866 by John Langdon Down (for whom the syndrome is now named).

There can be great variability in the severity of symptoms. Typical features 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 speech and symbolic thought.

In 1959 Prof. Jérôme Lejeune and Coll. 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".



Paris, 1969 – Prof. Maria Zannotti (second from the right) in a meeting with the group of Prof. Jérôme Lejeune (seated in the middle).

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

2. Results to date (March 2018)

2.1 Creation of transcriptional maps for human brain, hippocampus, blood cells, heart and thyroid to study expression of human genes in detail, in particular those 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
https://www.ncbi.nlm.nih.gov/pubmed/27345625
https://www.ncbi.nlm.nih.gov/pubmed/28923001

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 the journal *Human Molecular Genetics*:

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

Moreover, using the same method, we have delimited the critical region for congenital heart defects in DS:

https://www.ncbi.nlm.nih.gov/pubmed/28648597

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 140 enrolled individuals with DS and 56 control subjects.
We have conducted for the first time an analysis of plasma and urinary metabolome in DS. The results have been published in February 2018 in the journal *Scientific Reports*, and show that in DS there are specific anomalies of the metabolism:

https://www.ncbi.nlm.nih.gov/pubmed/29445163

3. New goals for 2018-2020

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 gene candidates as essential for DS manifestation. In particular, in collaboration with Prof. Patrick Harrison, University College Cork, Ireland, we will use the CRISPR/Cas9 system to remove the HR-DSCR from trisomy 21 cells (fibroblasts and lymphoblastoid cell lines), in order to identify changes in the metabolome specifically associated with this region. The NMR-detectable part of the metabolome will be studied in collaboration with Prof. Paola Turano, University of Florence.

3.2 Metabolomic profiling in biological samples from subjects with DS. We will continue 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.

"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/

4. 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

5. 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	Researcher "RTD-A"
Allison Piovesan	Biotechnology	Post-doc
Maria Caracausi	Biotechnology	Post-doc
Francesca Antonaros	Biotechnology	Post-Degree Fellow
Elena Cicchini	Biotechnology	Post-Degree Fellow
Gabriella Mattei		Laboratory technician

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

5.1 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

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

Prof. Paola Turano
Center of Magnetic Resonance (CERM), University of Florence, Florence, Italia

Prof. Patrick Harrison
Physiology Department, University College Cork, Cork, Ireland

Prof. Silvia Lanfranchi
Prof. Renzo Vianello
Department of Developmental Psychology and Socialization, University of Padova, Italy

Prof. Annalisa Radeghieri
Department of Molecular and Translational Medicine, University of Brescia, 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 raise about 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 fellowships.

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

For the period **Summer 2018 - Summer 2020**, based on the new objectives indicated above, we estimate a cost of **102.000 Euro/year** (**70.000** Euro/anno for three fellowships and **32.000** Euro/year for the expenses of the Laboratory).
In addition, a **laminar flow cabinet** (estimated cost **7,000** Euro) and an **electroporator** (estimated cost **13,000** Euro) will be needed for CRISPR/Cas9 experiments.

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

Molecular Biology / Accessory Expenses	Euro
Consumable and reagents	2,500.00
Tips, test tubes	2,500.00
Reagents for PCR, gel and purification of DNA	3,300.00
DNA sequencing	1,200.00
Reagent for in vivo cloning	3,000.00
RNA and cDNA	3,500.00
Reagents for Northern-blot	2,500.00
Reagents for "Real-Time" PCR	2,500.00
Reagents for functional assays	1,200.00
Hardware/software for data analysis	2,800.00
Publication costs of results	4,000.00
Costs for missions/collaborations [Participation at congresses and work meetings, organization of seminars]	3,000.00
Total for one year	**32,000.00**

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, instruments, reagents and services needed for the experimental work and publication of results.

8. Bibliography

Caracausi M, Vitale L, Pelleri MC, Piovesan A, Bruno S, Strippoli P. A quantitative transcriptome reference map of the normal human brain. Neurogenetics. 2014; 15: 267-287.

Caracausi M, Rigon V, Piovesan A, Strippoli P, Vitale L, Pelleri MC. A quantitative transcriptome reference map of the normal human hippocampus. Hippocampus. 2016; 26: 13-26.

Epstein CJ. Down syndrome, trisomy 21. In: Scriver CR, Beaudet al. Sly WS, Valle D. Metabolic Basis of Inherited Disease. McGraw-Hill, New York, NY, 1989. Pp. 291-326.

Gardiner K, Costa AC. The proteins of human chromosome 21. Am J Med Genet C Semin Med Genet, 2006, 142C:196-205.

Gardiner KJ. Molecular basis of pharmacotherapies for cognition in Down syndrome. Trends Pharmacol Sci, 2010, 31:66-73.

Korenberg JR. Down syndrome: the crucible for treating genomic imbalance. Genet Med, 2009, 11:617-619.

Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongoliens. C R Hebd Seances Acad Sci, 1959, 248:1721-1722.

Lenzi L, Facchin F, Piva F, Giulietti M, Pelleri MC, Frabetti F, Vitale L, Casadei R, Canaider S, Bortoluzzi S, Coppe A, Danieli GA, Principato G, Ferrari S, Strippoli P. TRAM (Transcriptome Mapper): database-driven creation and analysis of transcriptome maps from multiple sources. BMC Genomics, 2011, 12:121.

Letourneau A, Antonarakis SE. Genomic determinants in the phenotypic variability of Down syndrome. Prog Brain Res, 2012, 197:15-28.

Mégarbané A, Ravel A, Mircher C, Sturtz F, Grattau Y, Rethoré MO, Delabar JM, Mobley WC. The 50th anniversary of the discovery of trisomy 21: the past, present, and future of research and treatment of Down syndrome. Genet Med, 2009, 11:611-616.

Patterson D. Molecular genetic analysis of Down syndrome. Hum Genet, 2009, 126:195-214.

Pelleri MC, Piovesan A, Caracausi M, Berardi AC, Vitale L, Strippoli P. Integrated differential transcriptome maps of Acute Megakaryoblastic Leukemia (AMKL) in children with or without Down Syndrome (DS). BMC Med Genomics. 2014; 7:63.

Pelleri MC, Cicchini E, Locatelli C, Vitale L, Caracausi M, Piovesan A, Rocca A, Poletti G, Seri M, Strippoli P, Cocchi G. Systematic reanalysis of partial trisomy 21 cases with or without Down syndrome suggests a small region on 21q22.13 as critical to the phenotype. Hum Mol Genet. 2016 Apr 22. pii: ddw116. [Epub ahead of print] PubMed PMID: 27106104.

Pritchard M, Reeves RH, Dierssen M, Patterson D, Gardiner KJ. Down syndrome and the genes of human chromosome 21: current knowledge and future potentials. Report on the Expert workshop on the biology of chromosome 21 genes: towards gene-phenotype correlations in Down syndrome. Washington D.C., September 28-October 1, 2007. Cytogenet Genome Res, 2008, 121:67-77.

Roizen NJ, Patterson D. Down's syndrome. Lancet, 2003, 361:1281-1289.

Roper RJ, Reeves RH. Understanding the basis for Down syndrome phenotypes. PLoS Genet, 2006, 2:e50.

Sinet PM, Allard D, Lejeune J, Jerome H. Letter: Gene dosage effect in trisomy 21. Lancet, 1975, 1:276.

Strippoli P, Pelleri MC, Caracausi M, Vitale L, Piovesan A, Locatelli C, Mimmi MC, Berardi AC, Ricotta D, Radeghieri A, Barisani D, Basik M, Monaco MC, Ghezzo A, Seri M, Cocchi G. An integrated route to identifying new pathogenesis-based therapeutic approaches for trisomy 21 (Down Syndrome) following the thought of Jérôme Lejeune, Sci. Postprint, 1(1): e00010