"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 Histology, 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. Introduction

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.
To our knowledge, the project constitutes the most extensive clinical-experimental scientific research on Down syndrome conducted in Italy and aimed at identifying a cure for intellectual disability caused by the presence of an extra chromosome 21. The project currently involves 140 children with trisomy 21 between the ages of 3 and 16, with possible repercussions on all people with trisomy 21 (38,000 only in Italy), which is the most frequent genetic anomaly in humans.

We are studying in detail the critical region of chromosome 21 associated with Down syndrome and the metabolome (the set of metabolites) of trisomic cell models and children with the syndrome that will also undergo accurate clinical and cognitive assessment. The amount of data thus obtained and correlated with each other will be useful to identify specific altered metabolic reactions that could become effective therapeutic targets for the treatment of intellectual disability of Down syndrome.

2. Results to date (December 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):


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:


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


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:

2.4 The use of a cellular model (trisomic and euploid fibroblasts) has allowed us to verify the effects of various forms of folate on the antiproliferative action of methotrexate (MTX), an antifolic agent to which cells of people with Down syndrome are hypersensitive. These data can be interpreted in the context of a specific metabolism model for trisomy 21. The article was accepted December 21, 2018 for publication in the Journal of Cellular Physiology.


3. New goals for 2019-2021

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:


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 (Unit of...
Histology, Embryology and Applied Biology, University of Bologna. In particular, the project will be carried out by:

<table>
<thead>
<tr>
<th>First and Last Names</th>
<th>Degree</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pierluigi Strippoli</td>
<td>Medicine and Surgery</td>
<td>Associate Professor</td>
</tr>
<tr>
<td>Lorenza Vitale</td>
<td>Medicine and Surgery</td>
<td>Researcher</td>
</tr>
<tr>
<td>Maria Chiara Pelleri</td>
<td>Biotechnology</td>
<td>Researcher &quot;RTD-A&quot;</td>
</tr>
<tr>
<td>Allison Piovesan</td>
<td>Biotechnology</td>
<td>Post-doc</td>
</tr>
<tr>
<td>Maria Caracausi</td>
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<tr>
<td>Francesca Antonaros</td>
<td>Biotechnology</td>
<td>Post-Degree Fellow</td>
</tr>
<tr>
<td>Elena Cicchini</td>
<td>Biotechnology</td>
<td>Post-Degree Fellow</td>
</tr>
<tr>
<td>Gabriella Mattei</td>
<td></td>
<td>Laboratory technician</td>
</tr>
</tbody>
</table>

The clinical referent of the research is Prof. Guido Cocchi, Operative Unit of Neonatology, Policlinic S.Orsola-Malpighi, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, in collaboration with Dr. Chiara Locatelli of the same Unit (Director: Prof. Giacomo Faldella).

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. 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

For the three-year period 2019 - 2021, on the basis of the new objectives indicated above, we foresee a cost of **120,000 euros / year** (75,000 euros / year for three scholarships or research grants and 45,000 euros / year for laboratory costs). Furthermore, it will be necessary to purchase an inverted fluorescence microscope (estimated cost **14,000 euros**) for experiments with the CRISPR / Cas9 system and a freezer -80°C (estimated cost **14,000 euros**) for the expansion of the sample bank organic.

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

<table>
<thead>
<tr>
<th>Molecular Biology / Accessory Expenses</th>
<th>Euro</th>
</tr>
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<tr>
<td>Consumable and reagents</td>
<td>3,500.00</td>
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<tr>
<td>Tips, test tubes</td>
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<tr>
<td>Reagents for PCR, gel and purification of DNA</td>
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<td>DNA sequencing</td>
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<tr>
<td>Reagent for in vivo cloning</td>
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<tr>
<td>RNA and cDNA</td>
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<td>Reagents for Northern-blot</td>
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<td>Reagents for &quot;Real-Time&quot; PCR</td>
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<tr>
<td>Reagents for cell culture and functional assays</td>
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<tr>
<td>Hardware/software for data analysis</td>
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<tr>
<td>Publication costs of results</td>
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</tr>
<tr>
<td>Costs for missions/collaborations [Participation at congresses and work meetings, organization of seminars]</td>
<td>3,000.00</td>
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<tr>
<td><strong>Total for one year</strong></td>
<td><strong>45,000.00</strong></td>
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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


