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

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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)

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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.

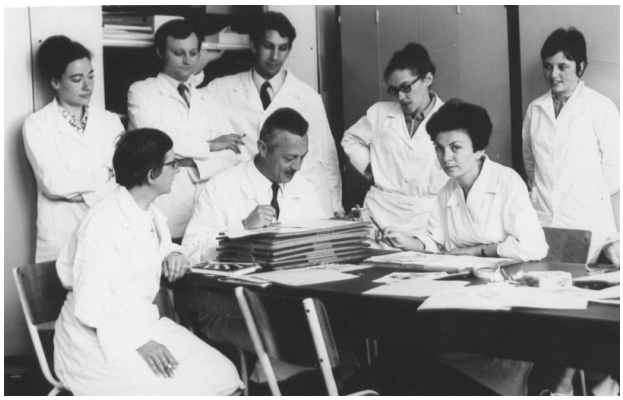
This chromosomal disorder causes a “syndrome”, a set of physical and mental "traits" originally described in 1866 by John Langdon Down (for whom the syndrome is now named).

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. However, it is common for children with DS to arouse a climate of affective intensity greater than normal so that some Authors have come to speak of a “kindness gene” or a “happy personality”.

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 Dr. Chiara Locatelli and 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 **230** 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.

In 2019 we wrote by invitation the chapter on genetics and genomics of Down syndrome in a new international book dedicated to the syndrome, summarizing the work done by Prof. Lejeune in this regard, and exposing our main results:
https://www.sciencedirect.com/science/article/pii/S2211609519300016
https://books.google.it/books?id=dtitDwAAQBAJ&pg=PA1&hl=it&source=gbs_toc_r&cad=3 - v=onepage&q&f=false

2. Results to date (January 2022)

2.1 HUMAN GENE EXPRESSION MAPS

We have created quantitative **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):

https://www.ncbi.nlm.nih.gov/pubmed/25185649
https://www.ncbi.nlm.nih.gov/pubmed/26108741
https://www.ncbi.nlm.nih.gov/pubmed/25476127
https://www.ncbi.nlm.nih.gov/pubmed/27345625
https://www.ncbi.nlm.nih.gov/pubmed/28923001
https://www.ncbi.nlm.nih.gov/pubmed/29740474

In 2021 we have published a transcriptome map of the blood from children with trisomy 21 by using the RNA-Seq method, in collaboration with the MAGI's Lab in Trento:

https://pubmed.ncbi.nlm.nih.gov/33933170/

2.2 DOWN SYNDROME CRITICAL REGION ON HUMAN CHROMOSOME 21

We have reanalysed all the 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*:

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

In 2019 a second article was published which confirms the relevance of the "critical region" for the diagnosis of Down syndrome on 10 new cases of partial trisomy 21, so to date, we do

not find any exception to the rule according to which in the absence of three copies of this particular region there is no diagnosis of Down syndrome.
This is in line with Prof. Lejeune's idea that on chromosome 21 there are few "culprit" genes and many "innocent" ones:

https://www.ncbi.nlm.nih.gov/pubmed/31237416

The "critical region" we identified on chromosome 21 is present only in humans and in the chimpanzee, and until 2021 it was considered silent (inactive), thus making it difficult to understand its function and its relationship with the features associated with SD. However, on December 8, 2021, our analysis of this region was published in *Frontiers in Genetics*, showing that it is actually active, and its DNA sequences are found to be expressed in the RNA transcripts of at least two genes:

https://pubmed.ncbi.nlm.nih.gov/34956324/

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 METABOLISM ALTERATION IN DOWN SYNDROME

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 227 enrolled individuals with DS and 77 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

A new article on the metabolomic profile in plasma was published on June 26, 2020, in *Scientific Reports*, confirming in a larger series that in Down syndrome there are specific metabolic abnormalities currently appearing not correlated with the degree of intellectual disability:

https://pubmed.ncbi.nlm.nih.gov/32591596/

In 2021 we published, again in *Scientific Reports*, results showing specific relationships between the monocarbon cycle (of which the folic acid cycle is part) and the cognitive abilities in children with Down syndrome:

https://pubmed.ncbi.nlm.nih.gov/33608632/

Some microRNA-containing blood plasma fractions also revealed specific differences compared to children without the extra chromosome, as shown in an article published in collaboration with the University of Brescia in the *International Journal of Molecular Medicine*:

https://www.ncbi.nlm.nih.gov/pubmed/31017260

The **cognitive data** collected thanks to the collaboration with the University of Padova also made it possible to study the relationship between the acquisition of the main developmental stages of the child and cognitive abilities, and the executive functions in children with DS, leading to the publication of two articles on these themes in 2021:

https://pubmed.ncbi.nlm.nih.gov/34069813/
https://pubmed.ncbi.nlm.nih.gov/34750907/

2.4 HUMAN CELLULAR MODELS OF TRISOMY 21

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 published in 2019 in the *Journal of Cellular Physiology*:

https://www.ncbi.nlm.nih.gov/pubmed/30667057

3. New goals for 2022

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 possibli induced pluripotent stem cell lines - iPSCs), in order to identify changes in the metabolome specifically associated with this region, in particular metabolic alterations related to the folic acid cycle.

The metabolites released by the cells in their culture medium will then be determined with ELISA assays or by metabolomic analysis of Nuclear Magnetic Resonance (NMR), in collaboration with the group of Prof. Paola Turano, University of Florence.

3.2 Metabolic profile in biological samples from people with Down syndrome. We will continue with the expansion of the biological bank by collecting urine, plasma and blood samples.

Following the hypothesis of Prof. Lejeune, the plasma analysis will focus in particular on the metabolites of the metabolic pathways known as the **one-carbon cycle** and the **folate cycle**, studying 30 children with Down syndrome and 30 control subjects. The aim is to identify specific alterations of the metabolism and to associate these alterations to certain genes located on chromosome 21, in particular in the "**critical region**", in order to identify possible targets of a therapeutic intervention.

Correlations will be sought between the results of the metabolomic analysis, the clinical characteristics and the results of the cognitive assessments.

The experimental methods are described in detail in the article we published describing the Project and in the bibliographic references cited there:

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

"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)

4. Chief Scientist Curriculum - Prof. Pierluigi Strippoli

Pierluigi Strippoli is Associate Professor of Applied Biology at the University of Bologna, Italy and has been teaching Genetics, Genetic Technologies, Genomics, Scientific Method in several academic courses. He leads the Laboratory of Genomics at the Department of Experimental, Diagnostic and Specialty Medicine (DIMES) of the same University.

He has been using molecular genetics and genomics technologies to study blood diseases, colorectal cancer and trisomy 21. His research group has identified one of the human chromosome 21 genes and he has conceived and developed several original computational biology tools for the analysis of the human genome, publishing 80 papers on international scientific journals.

Recently, Dr. Strippoli has focused on starting a systematic study of Down Syndrome by integrating clinical, biochemical, genetic and bioinformatic data in order to identify novel therapeutic targets for the intellectual disability associated with this form of trisomy.

The complete curriculum is available here:

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 Molecular Genetics, Department of Experimental, Diagnostic and Specialty Medicine (Director: Prof. Mauro Gargiulo from November 2015 to April 2021, Prof. Gianandrea Pasquinelli from May 2021), Unit 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	Associate Professor
Maria Caracausi	Biotechnology	Researcher "RTD-B"
Allison Piovesan	Biotechnology	Researcher "RTD-B"
Francesca Antonaros	Biotechnology	PhD Student
Beatrice Vione	Biotechnology	PhD Student
Giuseppe Ramacieri	Medicine and Surgery	PhD Student

Following the retirement of Prof. Guido Cocchi, the clinical referent of the research is currently Dr. Chiara Locatelli, Operative Unit of Neonatology (Director: Prof. Luigi Corvaglia), Policlinic S.Orsola-Malpighi, Department of Medical and Surgical Sciences (DIMEC), University of Bologna.

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.

6. Current National and International Collaborators

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

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

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

7. Funding

For the two-year period **2022 - 2023**, 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).

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	3,500.00
Tips, test tubes	2,500.00
Reagents for PCR, gel and purification of DNA	3,500.00
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 cell culture and folate assays	14,000.00
Hardware/software for data analysis	3,000.00
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	**45,000.00**

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. References

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