CTNNB1 Foundation

CTNNB1 Research Report

One step closer to a cure for CTNNB1 Syndrome.
Working Tirelessly. Including Every Child.

CTNNB1 Foundation The Gene Therapy Research Institute

CTNNB1 Foundation is a non-profit organization whose central purpose is to improve the lives of children diagnosed with life-threatening and life-limiting rare genetic mutations.

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The CTNNB1 Foundation was established on February 5, 2021, less than a year before the Foundation President's son, Urban, was diagnosed with CTNNB1 syndrome. Her strong desire and determination to help her son has grown to help all affected children.

The Foundation obtained the consent of the Ministry of Health of the Republic of Slovenia on the 10th of March 2021 and based on this consent it was entered into the register of institutions on the 7th of March 2021.

Our Mission

The CTNNB1 Foundation is a **non-profit organization** whose central purpose is to **improve the lives of children diagnosed with life-threatening and life-limiting rare genetic mutations.**

These, sometimes called orphan diseases, are simply not common enough to motivate for profit pharmaceutical companies to investigate further. In the end, it is usually the parents who fight alone to develop treatment solutions.

Our program includes the development of a **treatment for CTNNB1 Syndrome**. Findings from our studies will also be useful for research and treatment of other genetic diseases. We hope that further advances will lead to an **expansion of knowledge and also provide novel resources for other diseases** as well.

The motivation of this foundation is to make lives of the affected families at least a little easier. It involves **raising funds** and **financing activities to develop gene therapies** for children diagnosed with rare genetic diseases.

Our goal is to maintain hope and prove that, given time and sufficient funding, we will be able to cure or at least alleviate the symptoms of other rare genetic disease that are potential candidates for gene therapy.

The timing of therapy is very important. For parents, the clock is ticking. However, research takes time – or more importantly, – research should take time. **We want to follow all the necessary testing to make sure that the therapy is safe and effective.**

This foundation is currently funded by the founder's family, who has dedicated a sufficient amount to get this project started, but not enough to continue. For this reason, we are trying to obtain additional funds to finance the research and eventually develop a treatment for Urban and other children with the same diagnosis.

Let's step together and help our children on their way to a healthy future.

Team #CTNNB1

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Samo Miroševič, board member
Petra Prunk, MD, board member
Tanja Lavrič, MD, board member
Biserka Božičnik Križanec, treasurer

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CTNNB1 Foundation

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Edwin Chan, PhD, School of Life Science Hong Kong

School of Life Sciences
The Chinese University of Hong Kong

Damià Tormo, PhD, Columbus Foundation
Javier Garcia, PhD, Viralgen

fundación columbus

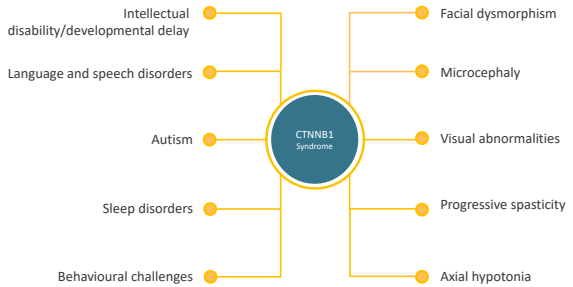
VIRALGEN

CTNNB1 Syndrome

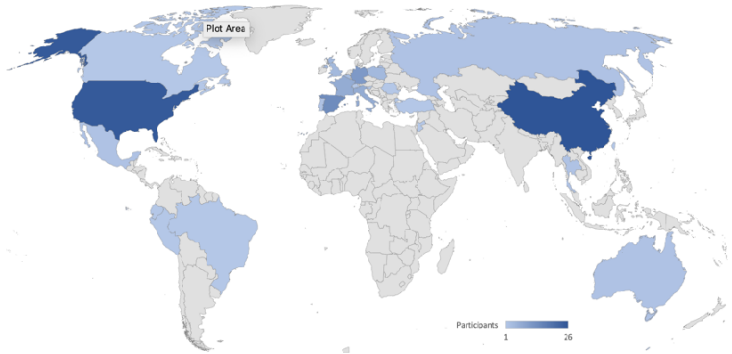
CTNNB1 Syndrome is a **debilitating disease**, primarily associated with the **developmental delay and intellectual disability.**

Children with CTNNB1 may also face other developmental issues and symptoms including **progressive microcephaly** (smaller head); **speech impairment**; **low muscle tone in the trunk** and **altered tone in the legs** (making walking difficult); **vision difficulties**; and skin, hair and mild **facial anomalies**.

The CTNNB1 gene codes for a protein called **beta-catenin** which plays **an important role in sticking cells together** (cell adhesion) and in **communication between cells.** The gene is important in the development and maturation of the brain. **Loss of its function** causes learning and memory problems.



Although there are currently **400 known children worldwide**, there are likely many more who have not been diagnosed. Two recent article found mutations in the **CTNNB1 gene** as the **most common cause of misdiagnosed cerebral palsy**[1,2].



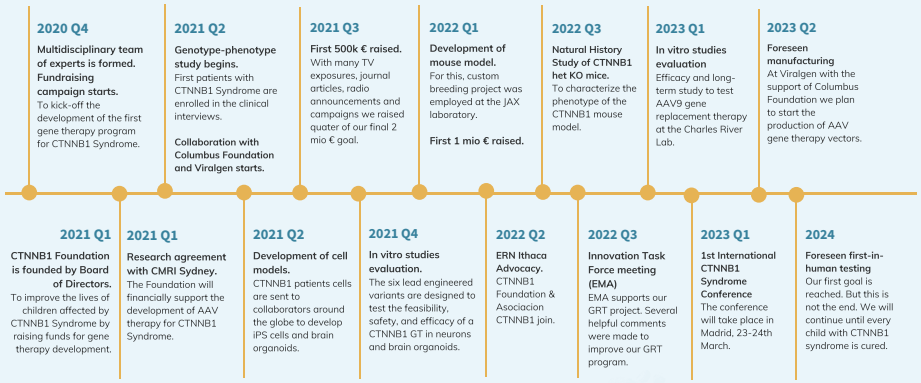
Patients with CTNNB1 are scattered around the world. Our Genotype-phenotype correlation study enrolled 125 patients (31.3% of 400 known).

[1] Moreno-De-Luca, A., Millan, F., Pesacreta, D. R., Elloumi, H. Z., Oetjens, M. T., Teigen, C., ... & Martin, C. L. (2021). Molecular diagnostic yield of exome sequencing in patients with cerebral palsy. Jama, 325(5), 467-475.

[2] Jin, S. C., Lewis, S. A., Bakhtiari, S., Zeng, X., Sierant, M. C., Shetty, S., ... & Kruer, M. C. (2020). Mutations disrupting neuritogenesis genes confer risk for cerebral palsy. Nature genetics, 52(10), 1046-1056.

Key Achievement

Here we present our key achievements from the time our foundation was founded until we foresee the start of the clinical trial. **We are very proud** that the we were able **to do so much in a such a short amount of time.**



We are excited to see our kid's future. While we anticipate to reach our goal when first in-human testing starts, we **will not stop there**. There are many more kids diagnosed worldwide and many more that will be born in the future. We feel it **is our responsibility to take care of every child with CTNNB1** Syndrome.

We won't stop until every child with CTNNB1 Syndrome is cured or treated.

Fundraising Efforts

The CTNNB1 Foundation was established to raise research funds for treatment of the CTNNB1 syndrome. The CTNNB1 Foundation is proud to manage these funds and use them for research projects that **will lead to treatments and cure for CTNNB1 syndrome.**

An additional grant of 300,000 euros from the Slovenian Research Agency was **awarded to the Slovenian research team** to continue the genotype-phenotype study (Damjan Osredkar, MD, PhD) and to test RNA and DNA therapeutic approaches (Roman Jerala, PhD).

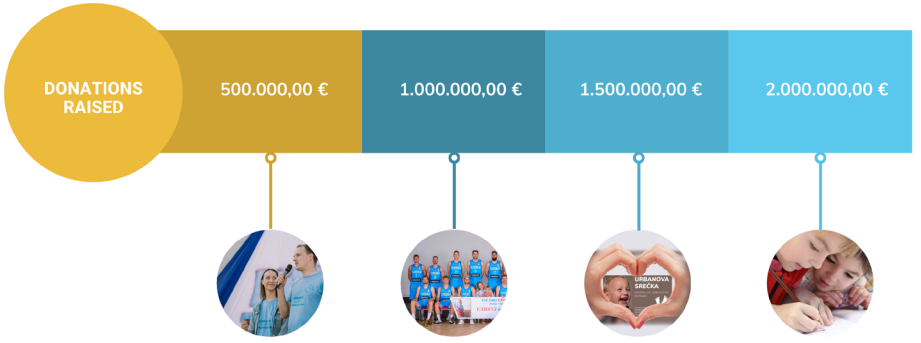
As of the end of November 2022, **we have raised 1.4 million euros** in donations, which is still short of **the required 2 million euros** required to bring the first therapeutic candidate to be ready for the clinical trial.

CTNNB1 Foundation

Palčica Pomagalčica

Fundraising Summary

FUNDRAISING GOAL
2.000.000,00 €



GOAL REACHED: NOV 2021

Our first half million was raised within 6 months of our first public interview. The CTNNB1 Foundation was able to raise this generous amount through various TV publications, the 'Slovenian Olympics for Urban' campaign and the 'Walking with Urban' charity event.

GOAL REACHED: MAR 2022

With the help of our generous donors, we raised our first million. Campaigns conducted to raise the funds include: 'Slovenian Basketball Players: Dončić for Urban' and several TV unveilings.

GOAL REACHED: DEC 2022

This is the amount to be raised by the end of 2022. At the moment, 1.4 million EUR has been collected. This amount was raised through campaigns such as 'Lottery tickets for Urban', 'Twins from Belgium' and 'Hockey players for Urban'.

GOAL REACHED: APR 2023

We expect to reach our fundraising goal of EUR 2,000,000.00 in the first quarter of 2023. Most of the money will be raised through 1% of the taxes that people living in Slovenia can donate to this program.

Research Activities

Since 2021, the CTNNB1 Foundation **has funded six research grants valued at over $700,000** (see Basic studies and treatment strategies). Led by our team of medical and scientific advisors, we seek to build on successful studies and **support risky but exciting new research** directed to improving the lives of affected patients.

We are also bringing together a larger group of researchers, neurologists, virologists, and other professionals at many meetings, including our **1st International Conference to be held March 23-24 in Madrid, Spain**.

This will allow them to share their findings and work together to determine the path forward for CTNNB1 research. Through these efforts, CTNNB1 hopes to help scientists **unravel the molecular mechanisms** of CTNNB1 syndrome and **develop strategies for effective medicines**.

To actively advance research toward our goals, CTNNB1 and its advisory boards are following a **roadmap** to explore and determine **which studies should be funded and explored**.

Our roadmap includes basic studies and **studies focused on finding best treatment solutions**. Basic studies include genotype-phenotype correlation study and biochemical characterization of mutations. It includes cell and animal model development and phenotyping. What we have observed from published data and our own data is that there is a lot of diversity in symptom severity.

Our goal is to find solutions for all children. Therefore, we are exploring all currently available technologies based on the gene therapy approach. **The most advanced approach is gene replacement therapy**, which is currently being tested for safety and efficacy.

In addition, **several RNA- and DNA-based technologies are being tested for a PoC**. CTNNB1 considers these goals equally important and carefully addresses each goal when allocating research funds. **Together, we strive for better treatments and a cure.**

CTNNB1 Basic Studies

To begin exploring different treatments options, basic research is needed to better understand patient symptoms, the impact of mutations, and the importance of cellular and animal models.

Study 1: Genotype-Phenotype Correlation

univerzitetni klinični center ljubljana
University Medical Center Ljubljana

- ClinicalTrials.gov Identifier: NCT04812119
- The main goal of the clinical trial is to reach out to the community of patients with CTNNB1 mutations and enroll as many patients as possible for a detailed clinical assessment of mutations in various domains.
- The evaluation will comprise of a questionnaire concerning the patient's history, prenatal and delivery risk factors, current medical issues.
- The study completed in Q4 2022 and enrolled 125 patients (31,3% of 400 known) from 24 countries.

Study 2: Biochemical characterization of mutations

School of Life Sciences
The Chinese University of Hong Kong

- The goal of this study is to identify correlations between particular genetic mutations and the various aspects of the disease phenotype.
- We are particularly interested in the effect of more prevalent mutations and mutations that have the potential to be amenable to treatment.
- This study evaluated 38 nonsense and frameshift variants and one missense variant. The study highlights that the pathogenic mechanism of CTNNB1 Syndrome is diverse which involves not only simple loss-of-function.

Study 3: Develop biological models and phenotype them

CHILDREN'S MEDICAL RESEARCH INSTITUTE
The Jackson Laboratory

- The goal of this project is to develop cell and mouse models to test novel treatment.
- Researchers at the Childrens Medical Research Institute are establishing phenotype of iPSCS dervied neurons and organoids.
- Natural history study at JAX lab of our developed one allele KO mice will be finished in 2023 Q1.

CTNNB1 Treatment Strategies

We are fighting for treatment solutions that could help all affected patients. While sever phenotype-associated mutations may require gene therapy (strategies 1 and 3), milder phenotype-associated mutations could be treated with less invasive treatments options (strategy 2).

Strategy 1: Gene Replacement Therapy

CHILDREN'S MEDICAL RESEARCH INSTITUTE

- Gene replacement therapy (GRT) is a one-time treatment that delivers a healthy copy of the gene to the cells with viral vectors.
- The majority of patients with a CTNNB1 syndrome have one mutated gene that causes loss-of-function of the protein called beta-catenin.
- Gene replacement therapy is a type of therapy where a functional copy of CTNNB1 is delivered to patient's cells through adeno-associated virus (AAV) delivery.
- Intended administration route is intrathecal administration.

Strategy 2: RNA-based Therapies

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- CTNNB1 Syndrome is an autosomal dominant condition. While one gene is mutated, the other one is healthy.
- The RNA therapies would enhance the wild type β-catenin from the healthy CTNNB1 allele.
- Inhibition of β-catenin degradation is currently explored by a) Skipping exon3 that encodes the regulatory region, b) Editing of β-catenin mRNA to mutate key phosphorylation residues that govern degradation, c) Downregulation of proteins involved in the β-catenin destruction complex.

Strategy 3: DNA-modification techniques

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- A "search and replace" gene editing method that can correct mutations in a precise way.
- Our researchers will work on the development of prime editing variation for genome editing for delivery via viral or nonviral (RNP, RNA) delivery.
- Currently, this is tested on a reporter and if successful on relevant cells harboring the specific CTNNB1 mutation.

Expense Report

Here we present the **CTNNB1 Foundation expense report for 2021-2022 and a forecast for 2023** based on projected spending.

More than 98% of the funds raised will go directly to our gene therapy program, including AAV therapy development, cell and animal model development and efficacy study, and planned toxicology and manufacturing of the AAV therapy.

	2021	2022	2023 (predicted)	TOTAL
EXPENSE				
Gene Therapy Program	167.718,95 €	123.707,08 €	-	291.426,03 €
Development of Cell and Mouse Model	29.783,12 €	22.427,27 €	-	52.210,39 €
Antisense Oligonucleotide Therapy	-	1.457,27 €	~30.000,00 €	31.457,27 €
Natural History Study of Mouse Model	-	55.338,25 €	-	55.338,25 €
Efficacy and Long-Term Safety Study	-	268.350,00 €	175.000,00 €	443.350,00 €
Toxicology	-	-	~100.000,00 €	100.000,00 €
Manufacturing			~1.000.000,00 €	1.000.000,00 €
Operating Expenses	5.485,59 €	6.218,22 €	~5.000,00 €	16.703,81 €
Others (translation, transport of the cell/animals)	30,00 €	5.217,85 €	~3.000,00 €	8.247,85 €
EXPENSES TOTAL	203.017,66 €	482.715,94 €	1.313.000,00 €	1.998.733,24 €

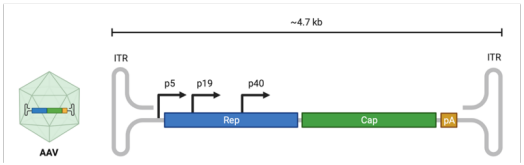
CTNNB1 Foundation – expense report

Treatment In The Preclinical Development: Urbagen

Urbagen is being developed to be a single-dose, preservative-free, sterile, intrathecal infusion of non-replicating, single-stranded AAV9 vector.

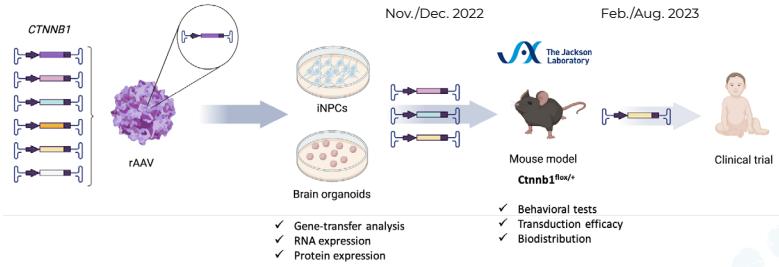
It contains the **human CTNNB1 gene**, which **encodes for the beta-catenin protein**, under the control of the **cytomegalovirus-enhanced chicken-beta-actin hybrid (CBh)/Neuron-specific synapsin 1 (SYN1) promoter.**

The size of the packaged single-stranded vector genome is ~4.7 kb.



Within our project, more than **a dozen therapeutic CTNNB1 gene therapy variants** with the CBh/SYN1 promoter have been designed, and some of them have passed early in silico evaluation and are being cloned for in vitro and in vivo evaluation.

These are the constructs that we are testing in iNPCs in order to triage the best ones for in vivo assessment. Within each candidate we are **systematically evaluating regulatory elements** in both the coding and non-coding components **to ensure both therapeutic efficacy and safety profiles of any therapeutic lead**.



CTNNB1 Foundation

TREATING EVERY CHILD. EVERYTIME.

The world we see is the world where parents would go to the doctor, the doctor would give them the child's diagnosis, and then he would give them a treatment.

We won't stop until this goal is reached.

Our kids don't have their voices. We are their only hope. Please consider supporting our kids.

Website: www.ctnnb1-foundation.org
Email: spela@ctnnb1-foundation.org

Consider donating to
CTNNB1 Foundation **TODAY**