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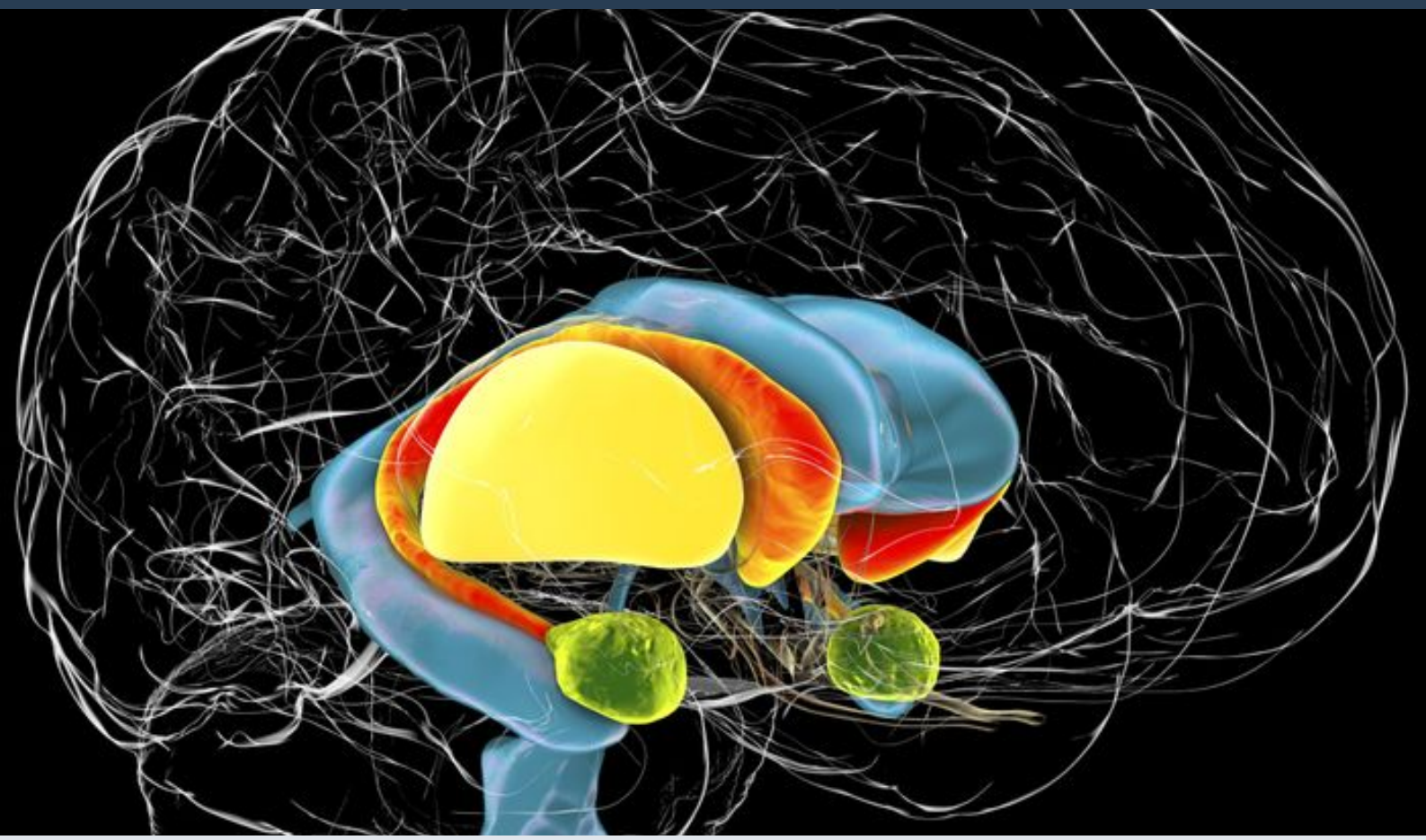
Huntington Disease Gene Repair Therapy

Adeno-associated virus CR SPR/Cas homology directed repair investigated to specifically repair Huntingtin repeats to healthy genotype

Reference: Huntington Gene Repair



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About **LMU Munich**

Ludwig Maximilians Universität München is the University in the heart of Munich. LMU is recognized as one of Europe's premier academic and research institutions. The LMU Munich community is engaged in generating new knowledge for the benefit of society at large

Background

Huntington's disease (HD) is characterized by a progressive loss of neuronal cells and the presence of misfolded Huntingtin containing aggregates. Regions affected by HD are the cortex with impairment of cortical development as well as hyperexcitability in the cortical neurons and the striatum with autophagy of its spiny neurons induced by reduced BDNF transport from the cortex. However, the precise mechanisms of this disastrous disorder are unknown and a therapy is currently impossible, which is also the consequence of missing reliable model systems. Brain organoids derived from human induced pluripotent stem cells (hiPSC) have the potential to fill this gap as these "mini brains" are miniature organs with anatomical features that resemble the real human brain. Cerebral organoids (COs) consist of 3D neuronal tissue including neurons, glia cells, and progenitors and reproduce the cortical plate like the 6 fold layering of the human cortex. Recently, hiPSCs have been used to differentiate into the developing human striatum including electrically active neurons. The assembly of these organoids with cerebral cortical organoids correspond to a very promising human model to investigate HD and to test therapy approaches. One of the best strategies to cure HD would be the repair of the mutant Huntingtin to its wildtype genotype.

Tech Overview

Within this approach LMU Munich researchers will use HD patient (and control) derived induced pluripotent stem cells to differentiate cortico striatal organoids (CS Ass) corresponding to a human "mini brain" model mimicking many features of the real human brain. For the differentiation we will use three different HD associated cell lines with different repeat length including ND38547 (Q44), ND36999 (Q180) and corresponding wt control ND50078 (WT). CS Ass growth will be monitored and organoid sections are neurodevelopmentally characterized using PAX6 (neuronal precursor), bTubIII (pre mature neurons), and MAP2 (mature neurons) immunofluorescence analysis. Discussed HD pathway aberrations in mutant CS Ass in comparison to control with normal CAG repeat length will be studied in order to qualify the Cs Ass technology as an appropriate model for HD. A newly developed gene editing approach (The method of choice to knock in large inserts via CRISPR | bioRxiv) based on adeno associated viruses (AAV) and homology directed repair (HDR) will be applied to living mini brain tissue sections and the extent of mutantHTT > wildtypeHTT repair will be quantified. The successful approach might pave the way to the clinical application of an AAV mediated healing of HD ().

Further Details

- The method of choice to knock in large inserts via CRISPR | bioRxiv
- Gene edited fluorescent cerebral organoids to study human brain function and disease | bioRxiv

Stage of Development

The organoid technology including CRISPR/Cas9 homology directed repair gene editing, stem cell cultivation and sorting, organoid differentiation is fully established in our lab. HTT hiPSC lines are available and have been successfully used to differentiate cerebral organoids (Figure 1).

Benefits

- A new approach to repair the mutant huntingtin gene and thereby treat Huntington disease will be investigated
- Due to the use of a long homology repair template and an off target reduced eCas9 the approach is highly specific
- A reliable human tissue model will be used and qualified as HD model (cortico striatal organoids)

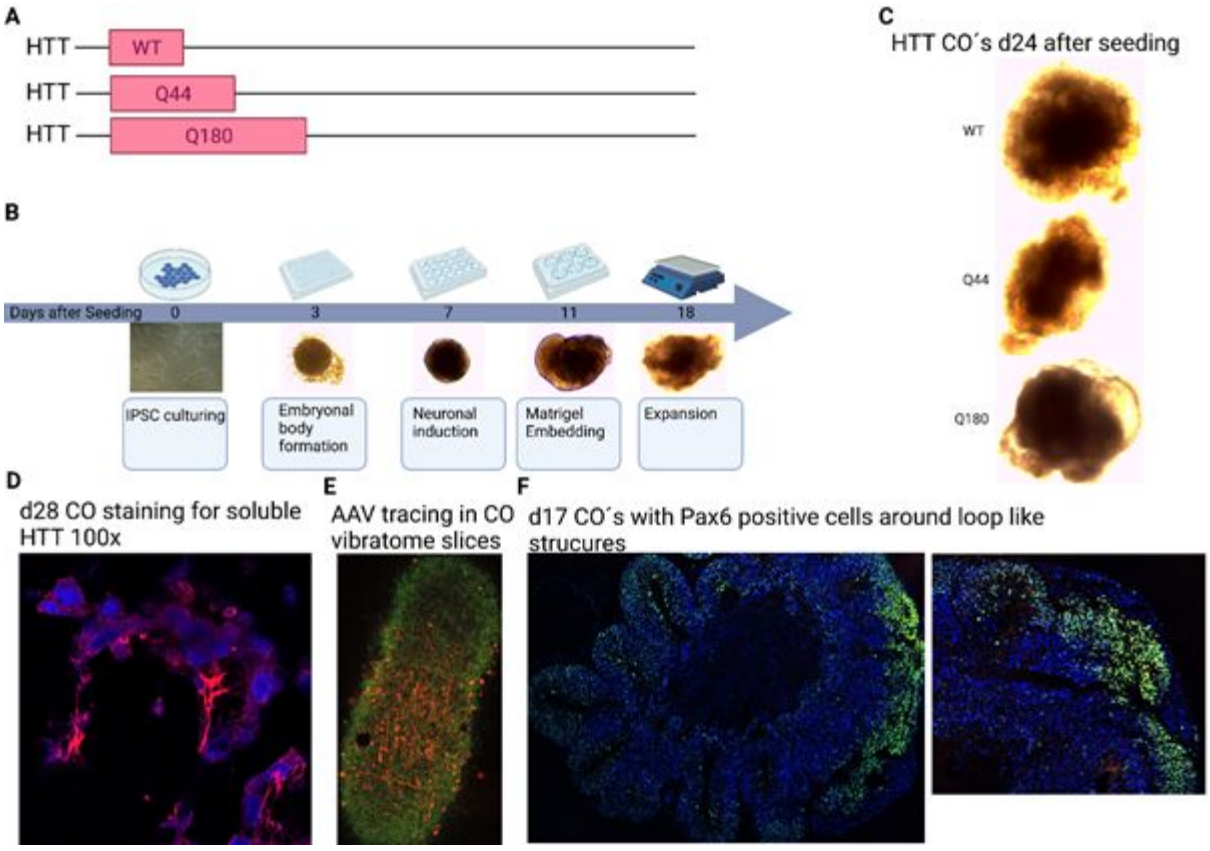
Applications

- Successful application of the AAV/CRISPR/HDR gene repair technology might pave the way to clinical application to repair huntingtin gene extensions to its wildtype genotype
- Successful application might stop further HD progression
- The gene repair approach might be applicable to a broad range of other genetic disorder
- The Cs Ass model might be applicable to study other human brain disorders

Appendix 1

Figure 1

HD cerebral organoids. (A) Three different hiPSC lines with indicated repeat lengths were used for cerebral organoid (CO) differentiation. **(B)** CO generation included different cultivation steps from hiPSC seeding to organoid expansion within 18d. **(C)** COs could be successfully generated for all three hiPSC lines. **(D)** Confocal immunofluorescence analysis using anti soluble HTT antibody revealed HTT expression in COs. **(E)** Adeno associated viruses (AAV) transducing a mTomato expression cassette were used to demonstrate high ratio of infected cells in CO slices. **(F)** CO's were fixed, stained for neuronal precursor cells (PAX6, yellow) and nuclei (DAPI, blue) and imaged by confocal microscopy and demonstrate loop like organization with circular precursor cells corresponding to a typical cortical plate organization found in mini brains.



For further information, please contact us.

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