Pluripotent stem cell lines from two patients with *COH1* gene mutations as the valuable *in vitro* model of Cohen Syndrome

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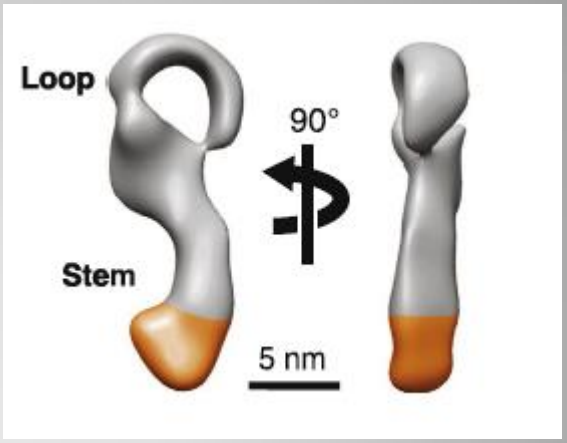
Cohen's syndrome (CS) is an autosomal recessive disorder caused by mutations in the *COH1 (VPS13B)* gene. CS is characterized by growth and mental retardation, microcephaly, and the development of autism spectrum disorders.

The COH1 protein is one of the key participants of the intracellular membrane transport system, the central part of which is the Golgi apparatus (GA). Recent studies have shown that mutations affecting GA proteins also result in the postnatal microcephaly associated diseases. GA is extremely important for the differentiation and functioning of postmitotic neurons. It plays a major role in determining and creating the cellular polarity necessary for the formation of highly specialized neurites – axons and dendrites.

The *COH1* gene has 62 exons and covers a region of approximately 864 kb on the human chromosome 8.

The product of the *COH1* gene is a peripheral membrane protein localized in GA and contributes to the structural maintenance and functioning of this complex. The supposed function of the COH1 protein is the regulation of vesicular transport and intracellular protein sorting.

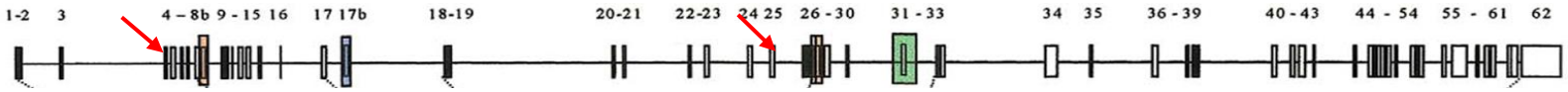


Joshua A et al, 2020

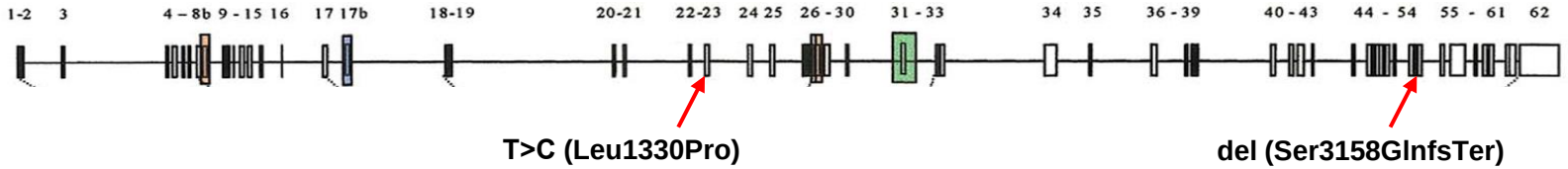
To study the effect of *COH1* gene mutations, we have obtained iPSC material of two patients with compound heterozygous mutations in the *COH1* gene:

*COH1*gene

864 Kb



Patient 1, (biopsy material - blood mononuclear cells) is a carrier of a compound heterozygous mutation (*COH1*-/-; chr8:g.100108653dup ENSP00000351346.2:p.Pro136ThrfsTer10; chr8:g.100494031G>T ENST00000358544.2:c.3870+1G>T)



Patient 2, (biopsy material - skin fibroblasts) is a carrier of a compound heterozygous mutation (*COH1*-/-; chr8:g.100514033T>C/ chr8:g.100844663_100844664del).

Patient 1

Source: *peripheral blood monocytes*
Description of a clinical case: *a 7-year-old girl. A presumptive diagnosis of autism spectrum disorder was made. The following clinical characteristics were noted: epicanthus, deep-set eyes, temporal narrowness, horizontal nystagmus, increased flexibility, hypotonicity, speech was represented by vocalizations.*

Patient 2

Source: *skin fibroblasts*
Description of a clinical case: *a 12-year-old girl. The following clinical characteristics were noted: microcephaly, weakness, fatigue, visual impairment and short stature. The girl had short neck, clinodactyly of 5th fingers, strabismus and severe hypermetropia with astigmatism, kyphosis of the thoracic spine, muscle hypotonia, mild intellectual disability with hyperactive disorder.*

For the skin fibroblasts of this patient, the following was revealed: swelling and vacuolization of Golgi Apparatus cisterns, as well as GA fragmentation, which is typical for the CS patients (Fig.1). In addition, in the CS fibroblast cytoplasm, there are a large number of electron-dense granules, vesicles, autophago- and lysosomes, as well as an arrangement of dense bundles of microfilaments associated with the nucleus (Fig.2).

Fig.1 control Patient2

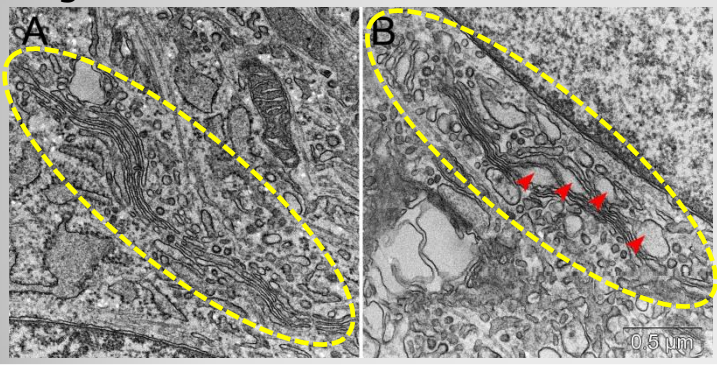
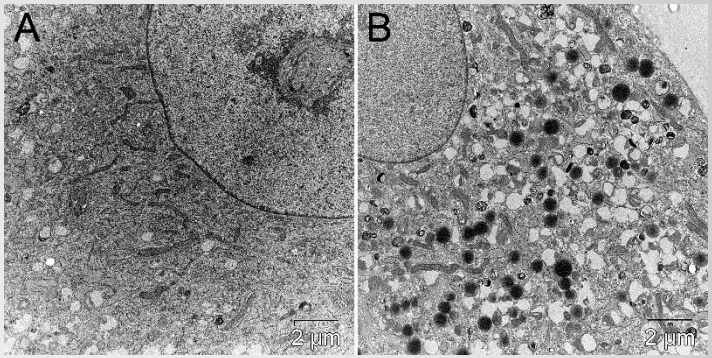


Fig.2 control Patient2



Derivation of pluripotent stem cell lines from two patients with *COH1* gene mutations

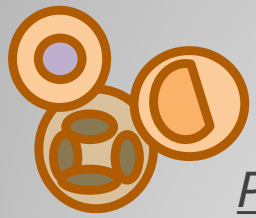
Patient 1

CS-MCF2
Skin fibroblasts
cell culture

Electroporation with episomal vectors

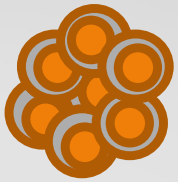
OCT3/4, SOX2, KLF4 , L-MYC LIN28, EBNA1
shRNA vs p53м

iPSC CS-MCF2



Patient 2

CS-MCM1
peripheral blood
monocytes



iPSC CS-MCM1

The iPSC lines from both patients have morphology of pluripotent cell, normal karyotype (46,XX) (Fig.3), express pluripotency markers (OCT4, NANOG), and surface markers SSEA-1 and TRA-1-60 (Fig.4). During differentiation in embryoid bodies, they contribute to Three germ layers (ectoderm, mesoderm, and endoderm).

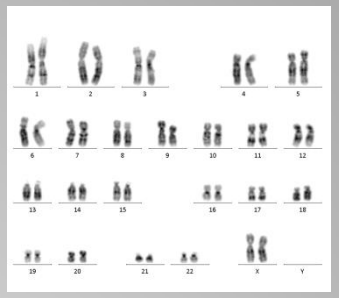


Fig.3

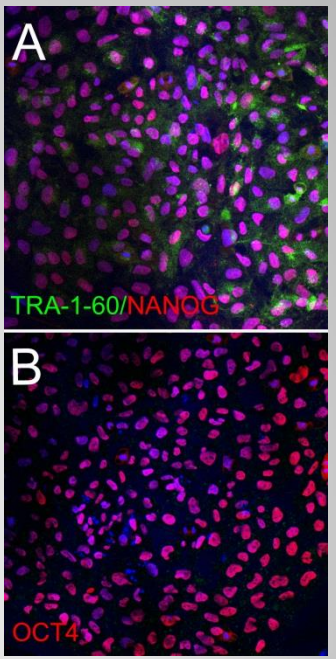
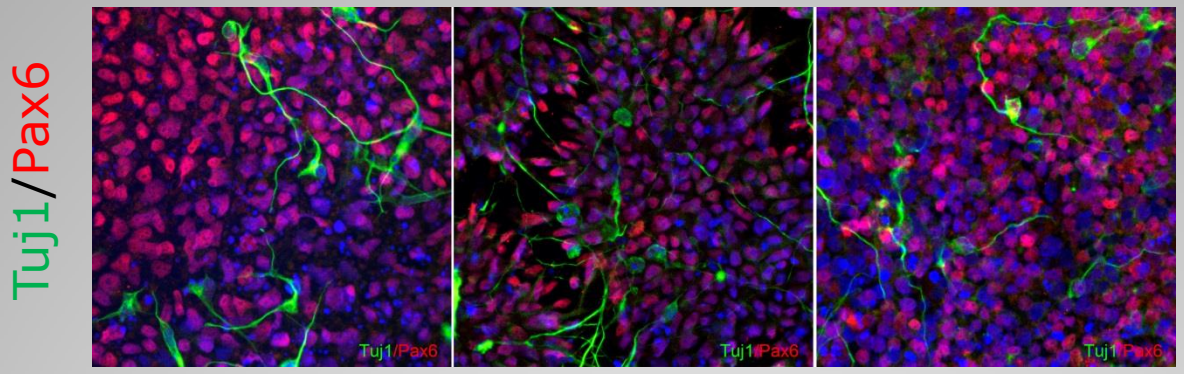


Fig.4

Fig.5 control patient1 patient2



To study the effect of mutations in the COH1 during neurogenesis, we checked the ability of iCS-MCF and iCS-MCM clones to differentiate into cortical neurons by double inhibition of the SMAD signaling pathway with SB431542 and Dorsomorphin. In 12 days both types of clones successfully expressed the primary neural progenitor cells marker (Pax6) and neuron-specific tubulin (Tuj1) (Fig.5)

There using will allow us to study the later stages of neurogenesis, in particular neuritogenesis, axons and dendrites formation, as well as the features of the structural organization of the GA and vesicular transport in the cell.

By studying the effect of mutations in the *COH1* gene that cause CS, we will be able to establish the role of this gene in the process of normal human neurogenesis.