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Analisi dei meccanismi neurobiologici di resistenza ai farmaci psicotropi in modelli cellulari innovativi

Coordinatore:

Prof. Massimo Gennarelli

Componenti

- **Massimo Gennarelli,** PhD, Professore Ordinario di Genetica Medica (MED/03)
- **Mariacristina Missale,** PhD, Professore Ordinario di Farmacologia (BIO/14)
- **Antonio Vita,** MD, Professore Ordinario di Psichiatria (MED/25)
- **Alessandro Barbon,** PhD, Professore Associato di Biologia Applicata (BIO/13)
- **Chiara Fiorentini,** MD PhD, Professore Associato di Farmacologia (BIO/14)
- **Alessandra Minelli,** PhD, RTDb Psicobiologia e psicologia fisiologica (M-PSI/02)
- **Federica Bono,** PhD, RTDa Farmacologia (BIO/14)

Partner

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- Villa Santa Chiara – Verona (Dott. Marco Bortolomasi)

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Obiettivo

- Generare iPSC (induced pluripotent stem cell), a partire da cellule del sangue di pazienti depressi e resistenti alla terapia farmacologica, differenziate in neuroni e analizzate mediante l'utilizzo di diverse piattaforme tecnologiche (imaging, genomiche e bioinformatiche) con lo scopo di definire i meccanismi neurobiologici alla base della resistenza ai farmaci antidepressivi.

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MAJOR DEPRESSIVE DISORDER (MDD)
1. Definition & prevalence

- MDD is a complex and highly heterogeneous psychiatric syndrome
- MDD is the most common psychiatric disorder and the second leading cause of disability worldwide
- The global lifetime prevalence has been estimated to be roughly 16% (WHO, 2003)
- Women have grater risk for MDD than men (PR W/M of 1.5-2.5)
- Its etiology and pathophysiology are poorly understood
- Only 1/3 of patients responds to the first antidepressant prescribed
- Another 1/3 develops Treatment Resistant Depression (TRD)

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MAJOR DEPRESSIVE DISORDER (MDD)
2. Diagnosis

DIAGNOSTIC CRITERIA FOR MDD (DSM-5, 2013) :

At least 1 of the 2 core symptoms:

1. Depressed mood and/or
2. Anhedonia (diminished interest or pleasure in all or most activities)

...must be observed in combination with at least 3 or 4 of the following additional ones:

3. Significant unintentional weight loss or gain
4. Insomnia or hypersomnia
5. Psychomotor agitation or retardation
6. Fatigue or loss of energy
7. Feeling of worthlessness or excessive guilt
8. Difficulty to think, concentrate or make decisions
9. Suicidal ideation or suicidal attempts

...in the same 2-week-period and cause clinically significant distress or impairment in social, occupational or other important areas of functioning

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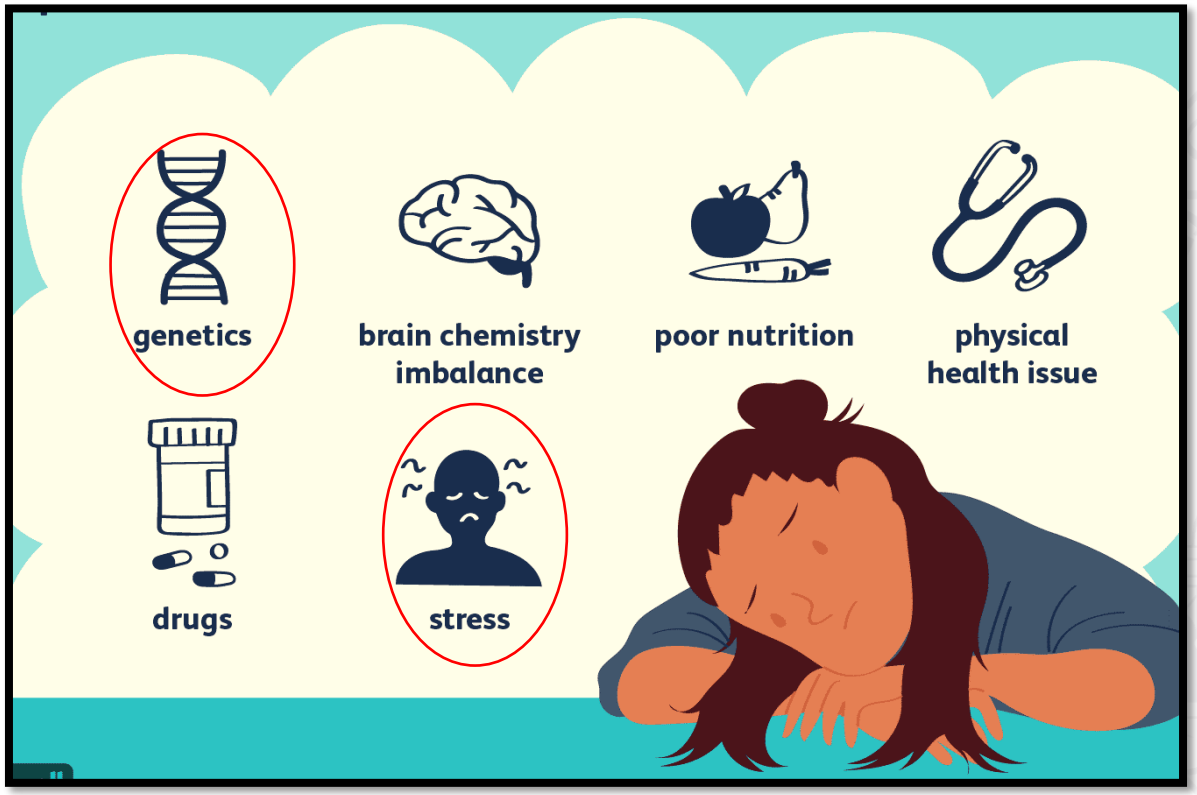
- NO OBJECTIVE DIAGNOSTIC TEST
- A WIDE VARIATIONS IN CLINICAL SIGNS AMONG PATIENTS

MAJOR DEPRESSIVE DISORDER (MDD)
3. Etiology



MDD is a multifactorial and polygenic disorder where cumulative interactions of genetics and stressful life events contribute to predispose an individual to develop this illness.

MAJOR DEPRESSIVE DISORDER (MDD)
5. PHARMACOLOGICAL TREATMENTS

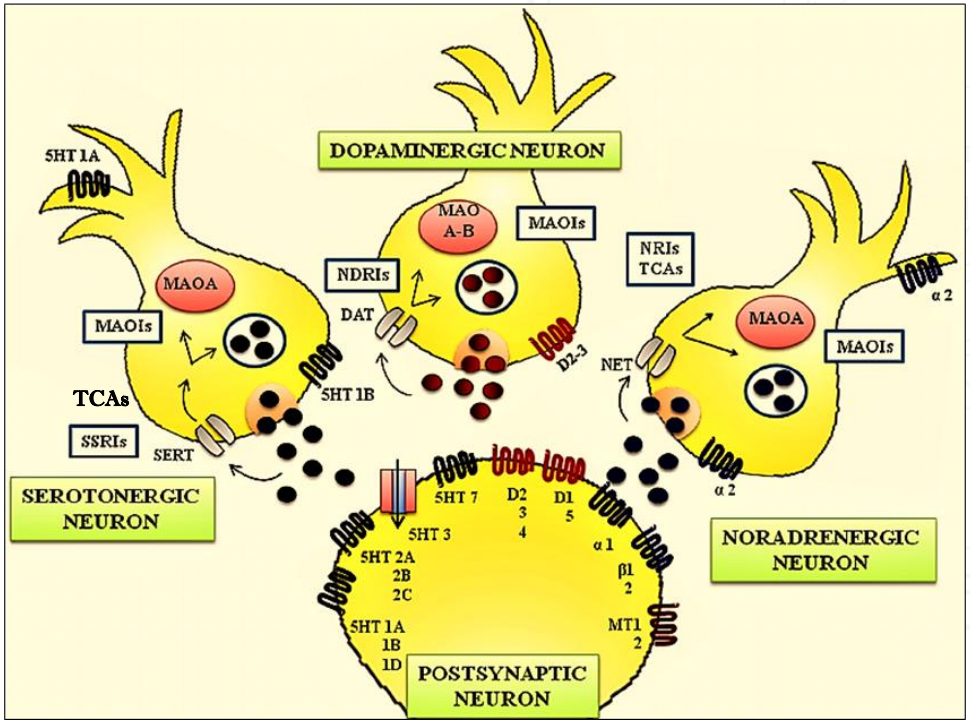
MONOAMINE HYPOTHESIS OF DEPRESSION

MDD is caused by altered production, release, tournover or function of monoamine neurotransmitters

Available drugs mainly target the monoamine neurotransmitter systems

But...

1. delayed therapeutic effect (2-3 weeks)
2. low rensponse rate



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MAJOR DEPRESSIVE DISORDER (MDD)
7. Treatment Resistant Depression (TRD)

- No single and unequivocal accepted definition of TRD
- TRD usually refers to the:

FAILURE TO ACHIEVE THE THERAPEUTIC RESPONSE AFTER AT LEAST TWO TRIALS OF DIFFERENT CLASSES OF ANTIDEPRESSANTS ADEQUATE IN DOSE AND DURATION

- Approximately 1/3 of patients develops TRD

The poor understanding of TRD mechanisms and its high incidence probably reflect the INTRINSIC BIOLOGICAL AND ENVIRONMENTAL HETEROGENEITY among TRD patients

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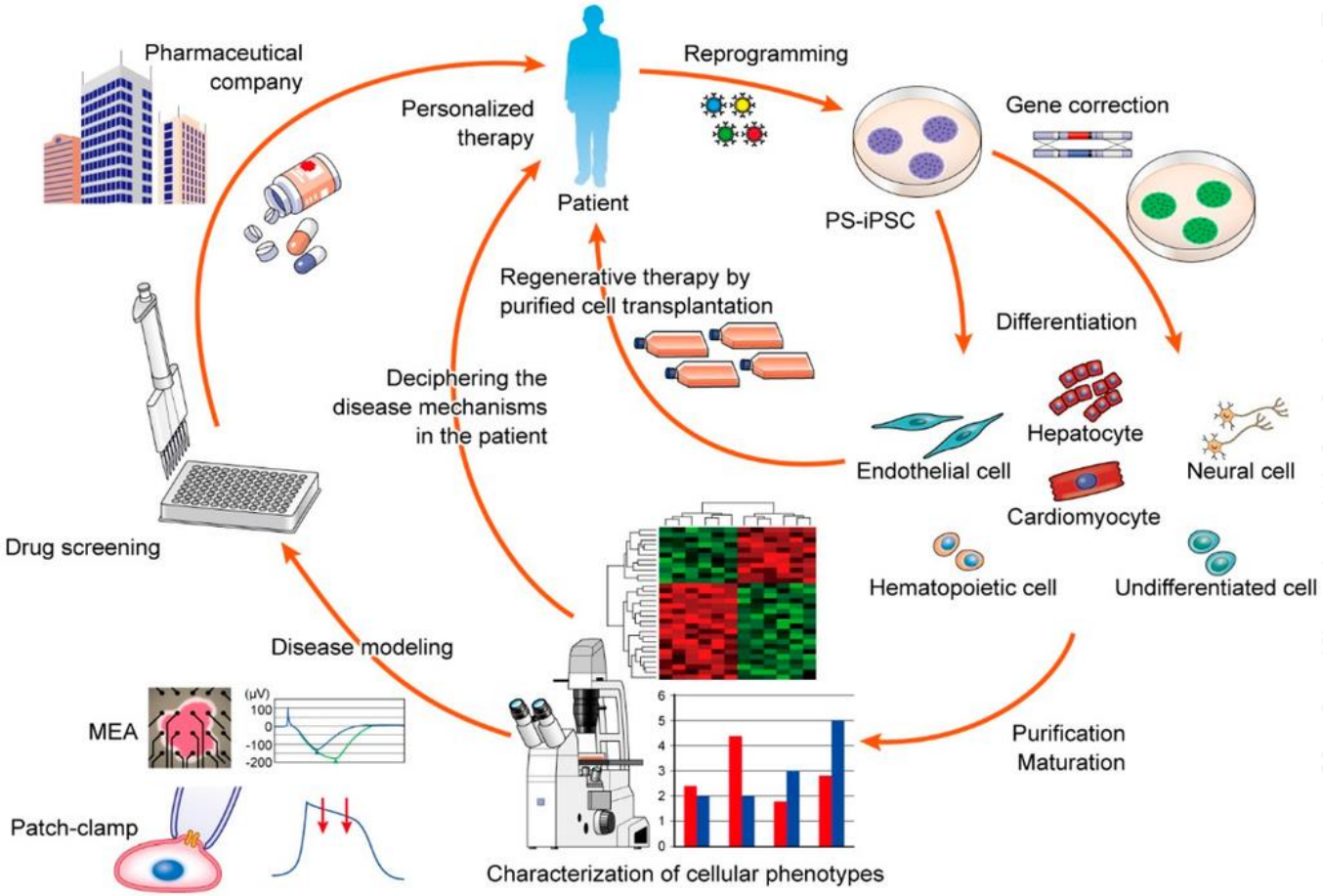
MAJOR DEPRESSIVE DISORDER (MDD)
8. Modeling MDD with hiPSCs



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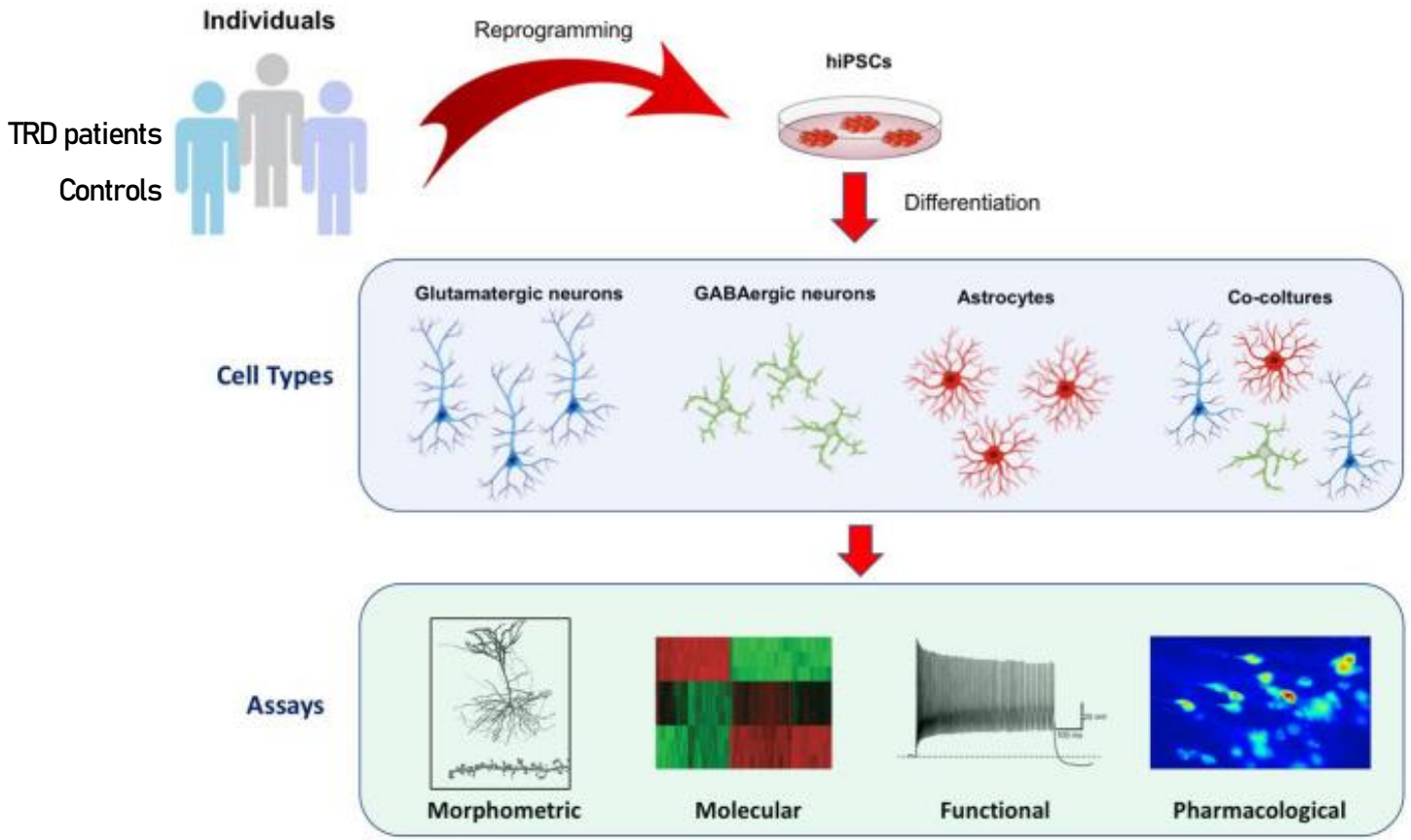
AIM OF THE PROJECT & EXPERIMENTAL PLAN



Using iPSCs-derived neurons to investigate the molecular mechanisms underlying Treatment Resistant Depression (TRD) focusing on BDNF pathway

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1. SUBJECTS ENROLLEMENT

PATIENTS

- 2 TRD female patients (P1 and P2)
- 2 TRD male patients

CONTROLS

- 2 female Healthy controls (C1)

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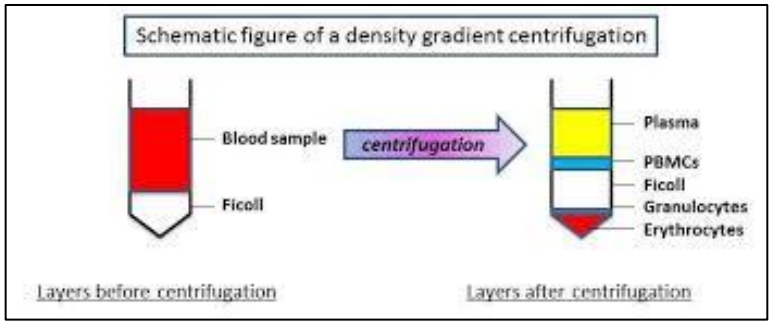
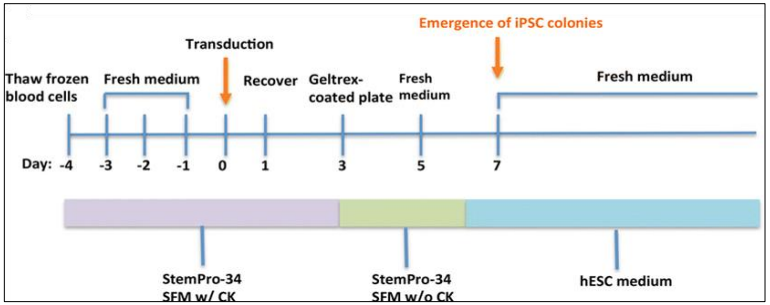
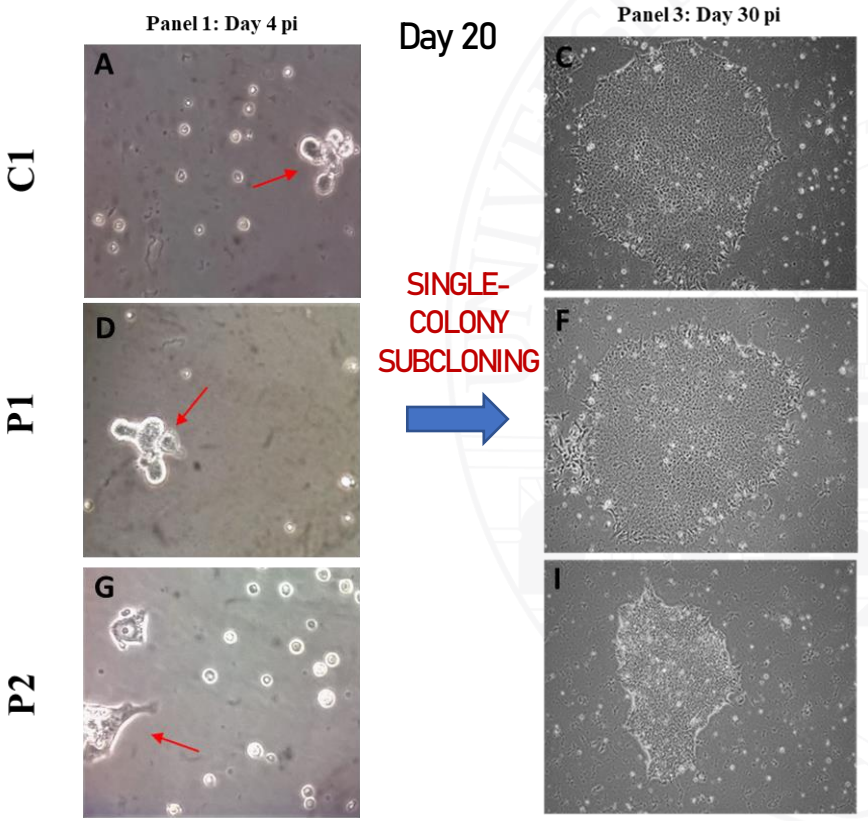
2. iPSCs GENERATION FROM PBMCs

PBMCs ISOLATION FROM PERIPHERAL BLOOD SAMPLES OF C1, P1 and P2



C1, P1 and P2 PBMCs REPROGRAMMING TO iPSCs WITH SENDAI VIRUS





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3. iPSCs CHARACTERIZATION

Contents lists available at ScienceDirect

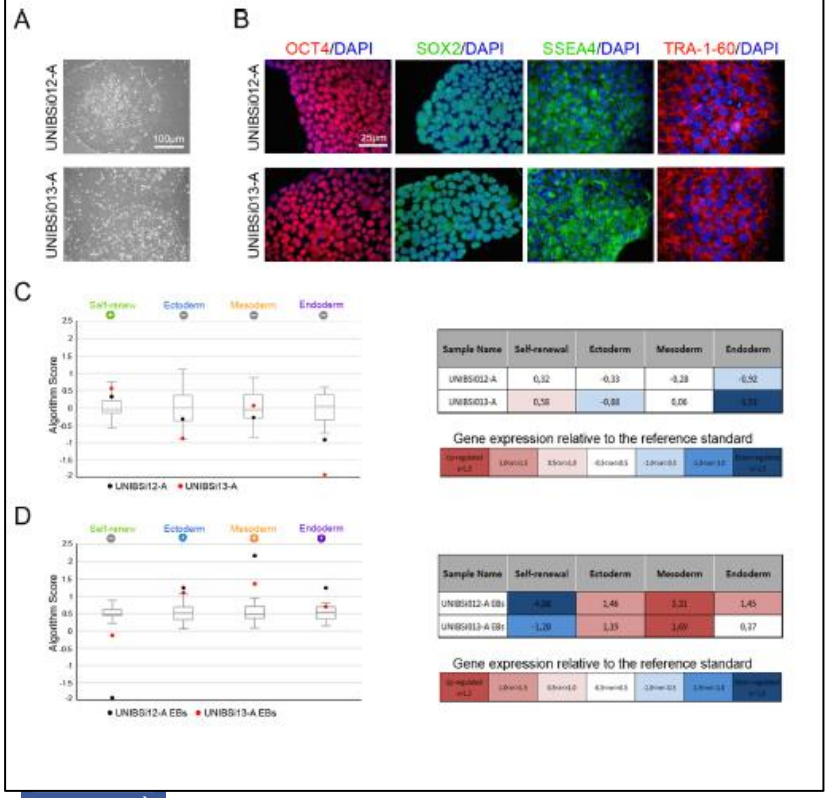
Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Multiple Cell Lines

Generation of two human induced pluripotent stem cell lines, UNIBSi012-A and UNIBSi013-A, from two patients with treatment-resistant depression

Federica Bono [a], Veronica Mutti [a], Giovanna Piovani [b], Alessandra Minelli [b,c], Jessica Mingardi [b], Adele Guglielmi [a], Chiara Fiorentini [a], Alessandro Barbon [b], Cristina Missale [a], Massimo Gennarelli [b,c,*]



Contents lists available at ScienceDirect

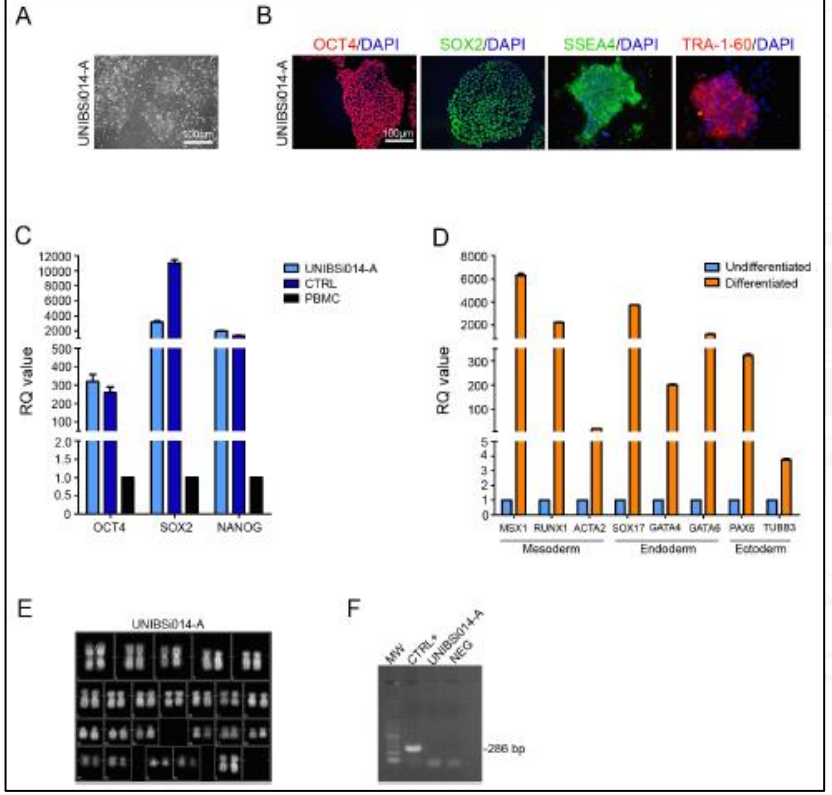
Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: Stem Cell Line

Establishment and characterization of induced pluripotent stem cell (iPSCs) line UNIBSi014-A from a healthy female donor

Federica Bono [a], Veronica Mutti [a], Giovanna Piovani [b], Alessandra Minelli [b,c], Jessica Mingardi [b], Adele Guglielmi [a], Cristina Missale [a], Massimo Gennarelli [b,c], Chiara Fiorentini [a,*], Alessandro Barbon [b]



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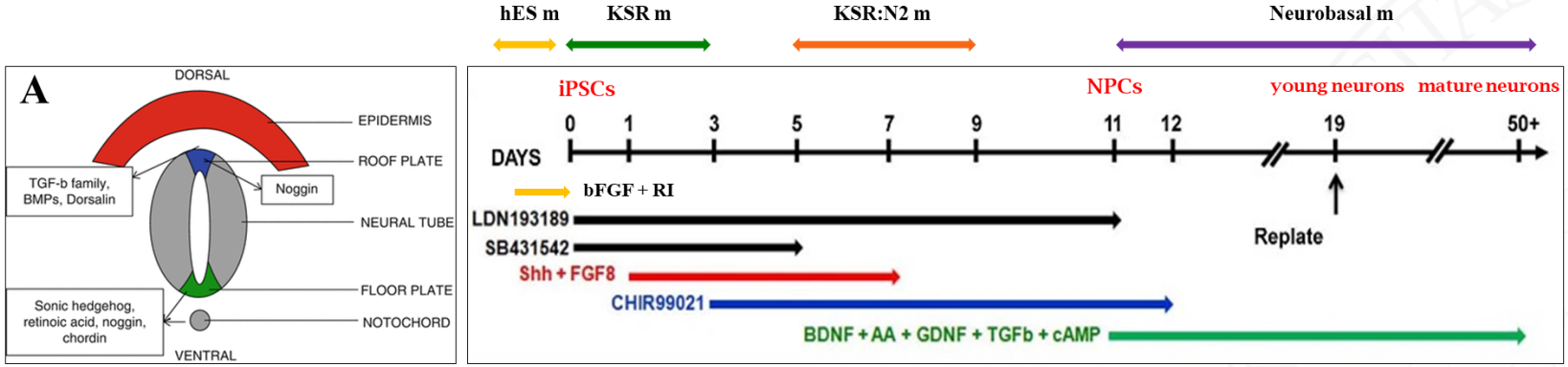
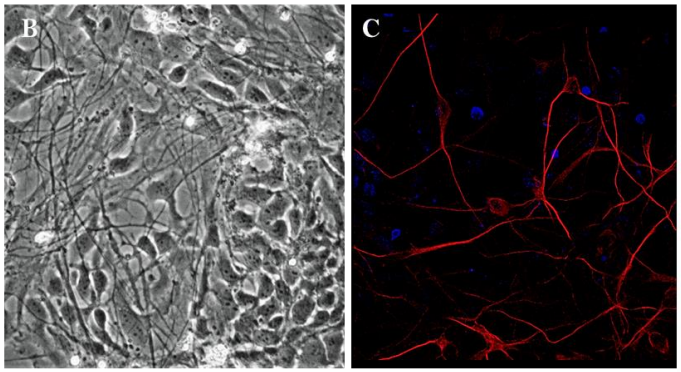
4. DIFFERENTIATION OF iPSCs toward NEURONAL cells

DUAL SMAD INHIBITION PROTOCOL FOR NEURONAL INDUCTION OF iPSCs (Kriks et al., 2011; Bono et al., 2018)

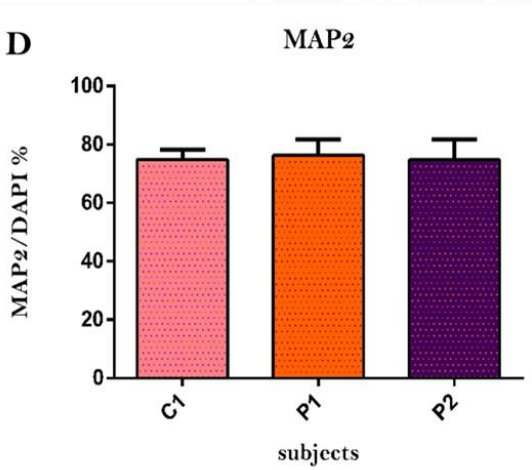


iPSCs-DERIVED NEURONS FROM HEALTHY CONTROL (C1) at day 25 (B) and day 50 (C)

MAP2/DAPI



NEURONAL YIELD VALIDATION in C1, P1 and P2 CULTURES



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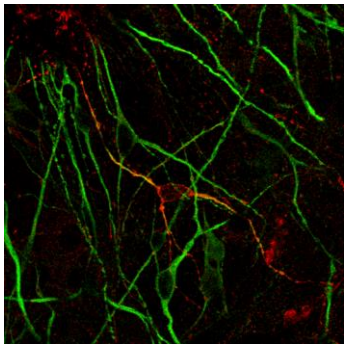
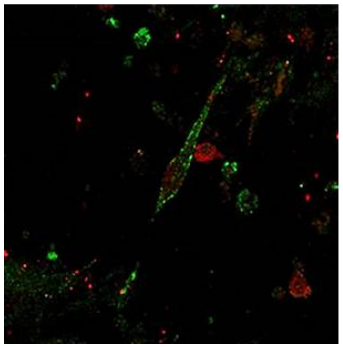
5. CHARACTERIZATION ON NEURONAL COLTURES (IHC)

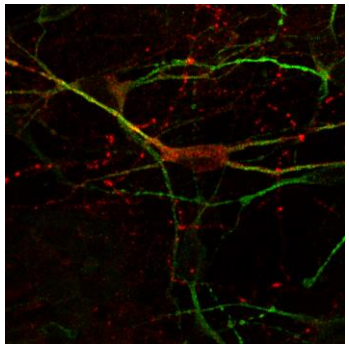
MAP2/TH



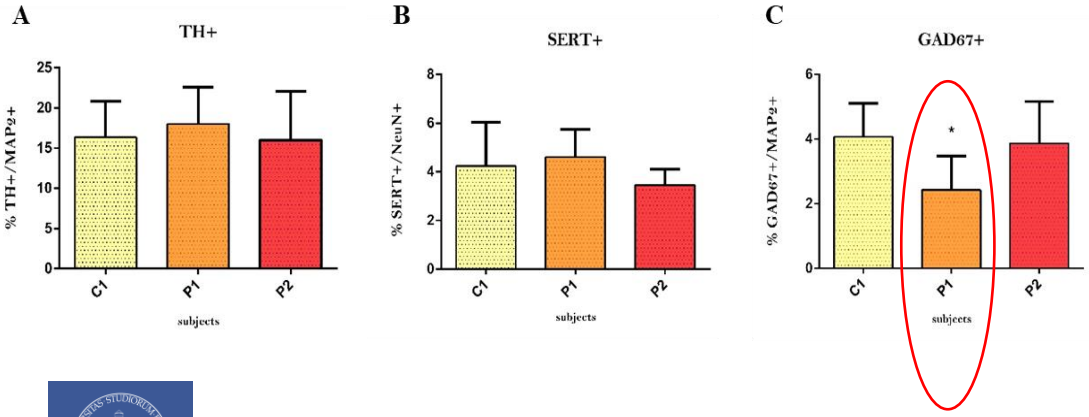
SERT/NEUN



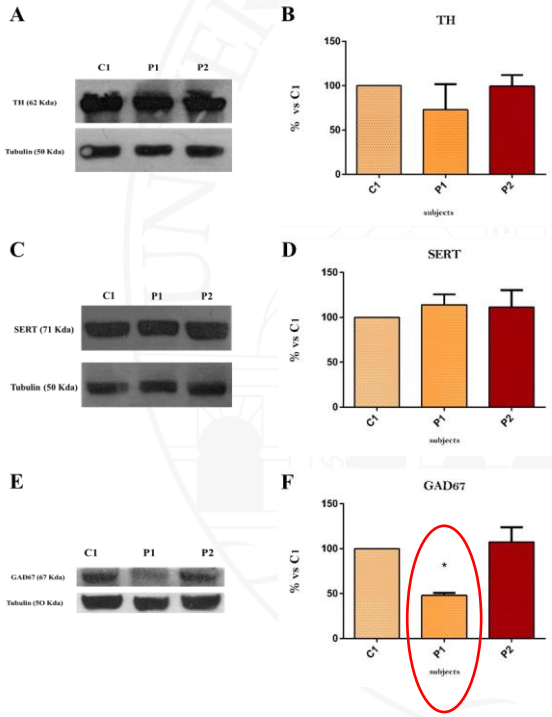
MAP2/GAD67



IF QUANTITATIVE ANALYSIS OF THE THREE MAIN NEURONAL SUBPOPULATION IN C1, P1 and P2 NEURONAL CULTURES



WB ANALYSIS OF THE THREE MAIN NEURONAL SUBPOPULATION IN C1, P1 and P2 NEURONAL CULTURES



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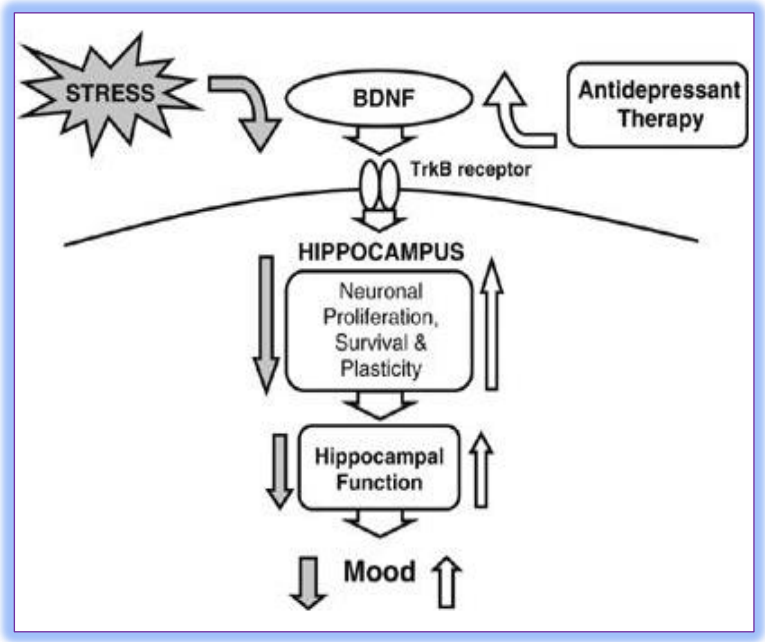
Altered neuroplasticity hypothesis: BDNF as a key transducer of antidepressants effect



Different classes of antidepressants increase the expression of BDNF in the main brain areas involved in the pathophysiology of depression

The neurotrophic hypothesis of depression proposes that MDD is associated with reduced brain BDNF levels, while the ability in increasing the BDNF/TrkB pathway likely represents the main molecular mechanism underlying the pharmacological action of the most class of antidepressant drugs

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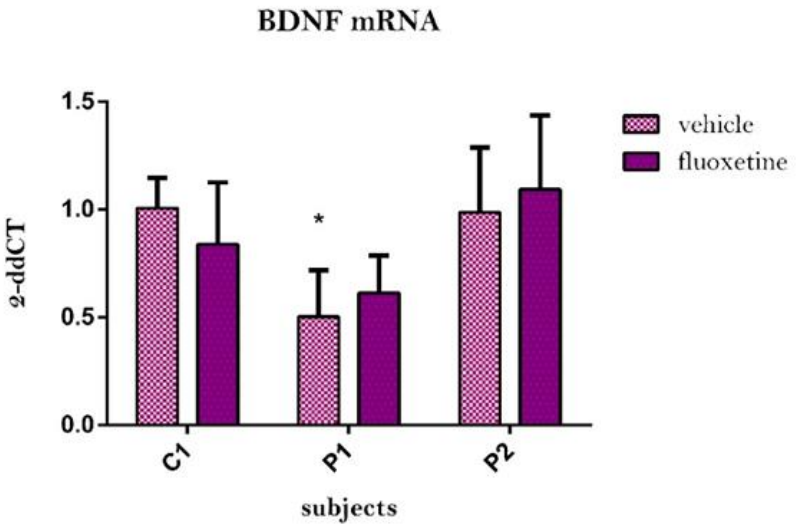
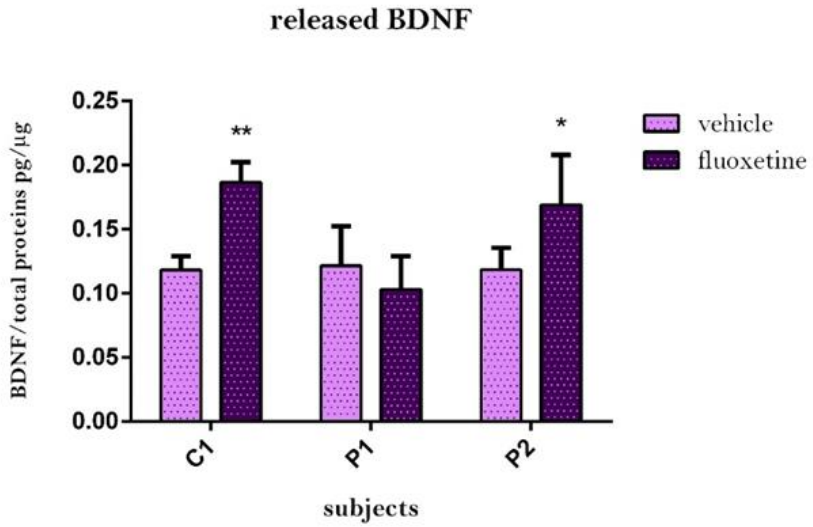
6. ANALYSIS OF THE BDNF PATHWAY

1. C1, P1 and P2 neuronal cultures were treated with SSRIs Fluoxetine
2. Analysis of the BDNF mRNA and protein

qPCR ANALYSIS OF BDNF mRNA LEVELS AFTER 48 h OF FLUOXETINE TREATMENT



ELISA ASSAY OF BDNF PROTEIN RELEASE AFTER 48 h OF FLUOXETINE TREATMENT



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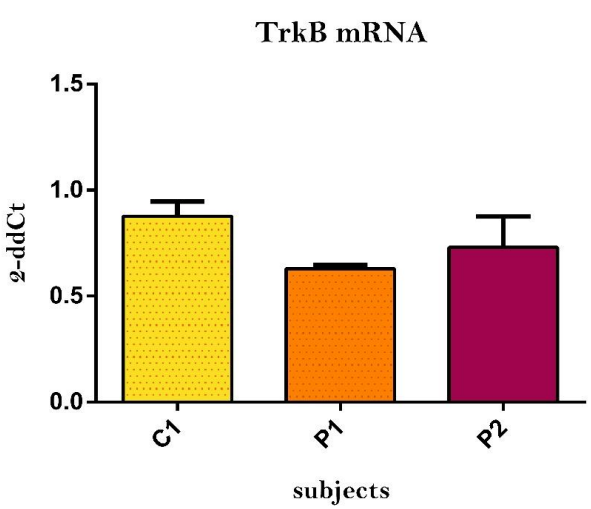
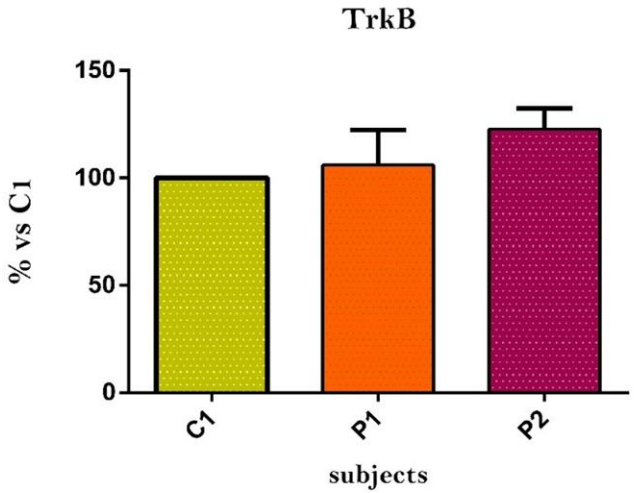
6. ANALYSIS OF THE BDNF PATHWAY

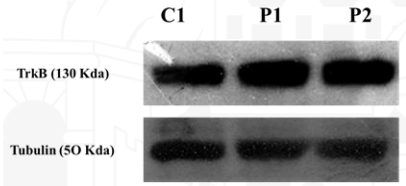
C1, P1 and P2 neuronal cultures analyzed in basal conditons for the expression levels of BDNF receptor TrkB (mRNA & protein)

qPCR ANALYSIS OF TrkB mRNA LEVELS IN BASAL CONDITIONS



WB ANALYSIS OF TrkB IN BASAL CONDITIONS





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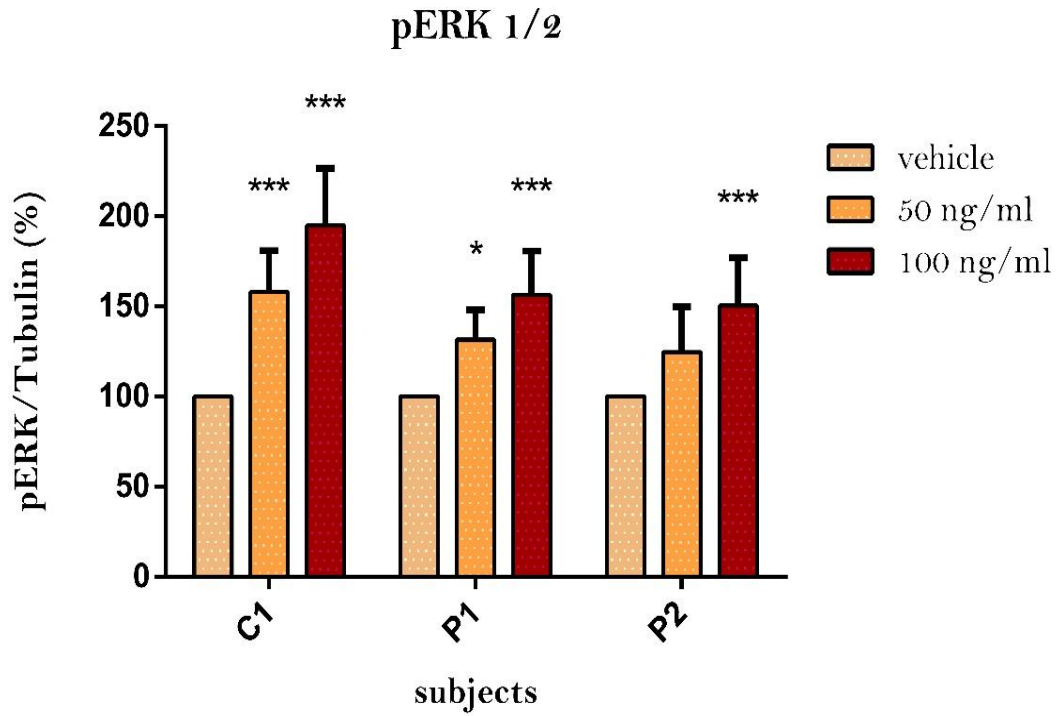
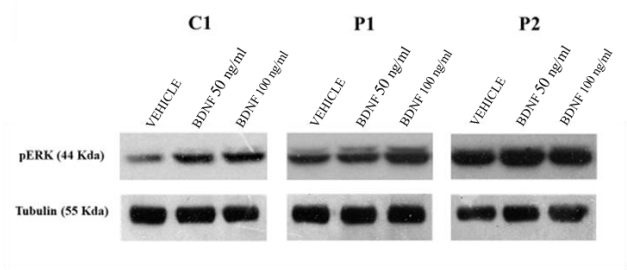
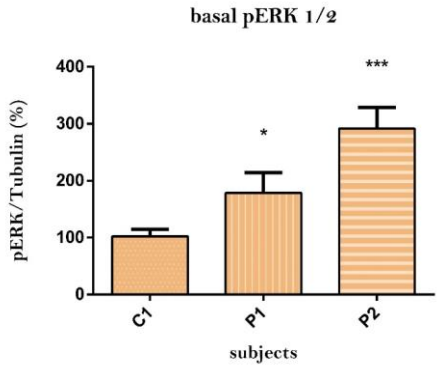
6. ANALYSIS OF THE BDNF PATHWAY

1. C1, P1 and P2 neuronal cultures were treated with two doses of BDNF
2. Analysis of ERK 1/2 activation by western blot







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CONCLUSIONS

Major results of this study were:

1) the quantitative analyses of the different neuronal populations at the end of the differentiation protocol, from hiPSC toward neurons, indicate that in one TRD patient (P1), the GABAergic neurons were significantly reduced compared to both control, and the P2 TRD patient.
2) a deficit in the BDNF/TrkB signaling has been evidenced in both the TRD patients, in line with the observation that the BDNF signaling pathways is crucially required for antidepressants action. The most relevant findings were that P1 neuronal cultures lost the ability to release BDNF following fluoxetine treatment while P2 neurons showed a reduced ability to respond to exogenous BDNF by activating TrkB-dependent Erk1/2 cascade.

The hiPSC technology could be a useful approach for identifying specific molecular abnormalities for each patient likely contributing to complex mechanisms that lead to resistance to antidepressant drugs.

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- Dr. Jessica Mingardi

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