Cri du Chat Induced Pluripotent Stem cells :New Frontiers In Disease Understanding

Khallafalla Siddig[1], Giovanna Piovani[1], Gaetana Lanzi[2], Rosalba Monica Ferraro[2], Stefania Masneri[2], Chiara Barisani[2], Eltahir Ali1,[2] Giulia Savio[1], Silvia Clara Giliani[2]

1 Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy , 2 A. Nocivelli Institute for Molecular Medicine, Italy

Background

➢ Cri du chat (CdC) syndrome is a genetic disease caused by partial or total loss of the short arm of chromosome 5. A group of genes (1.6 %) was classified as dosage sensitive leading to haploinsufficiency. **TERT**, **SEMA5A** and **CTNND2** are known to be expressed in the brain, neurons and the developing nervous system and play a role in neural migration.

➢ The function of most of the genes on **5p** are still unknown. From two CdC patients' peripheral mononuclear blood cells, we generated induced pluripotent stem cells (IPSCs) ; to obtain differentiated tissue with the same genetic background, to offer a valuable tool for studying this genetic disease and designing potential therapies .

Generation of IPSC clones & karyotyping

Figure 1

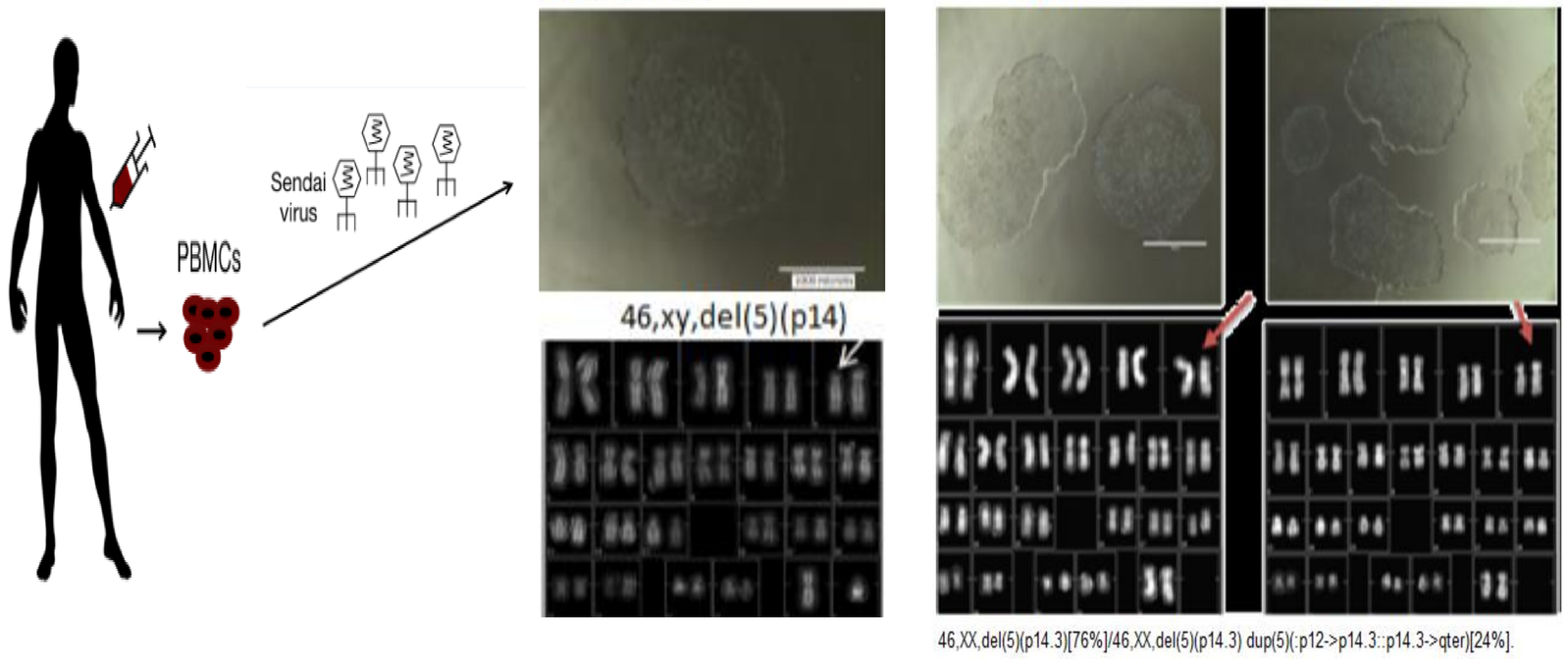

FIGURE 1. generation of CdC IPSC patient (1) UNIBSi004-A, from the peripheral blood mononuclear cells of a 35-year-old male affected by CdCS. The patient's karyotype shows a large deletion, from band p14 to the end of the short arm of one chromosome 5 (46,XY,del(5)(p14)) . **patient (2)** The Karyotype of **C6** show the normal 5del, while **C9** sample show complex karyotype with mosaicism ,The arrows show the abnormal chromosome 5 .

Charactrization of the generated IPSCs clones

Figure 2

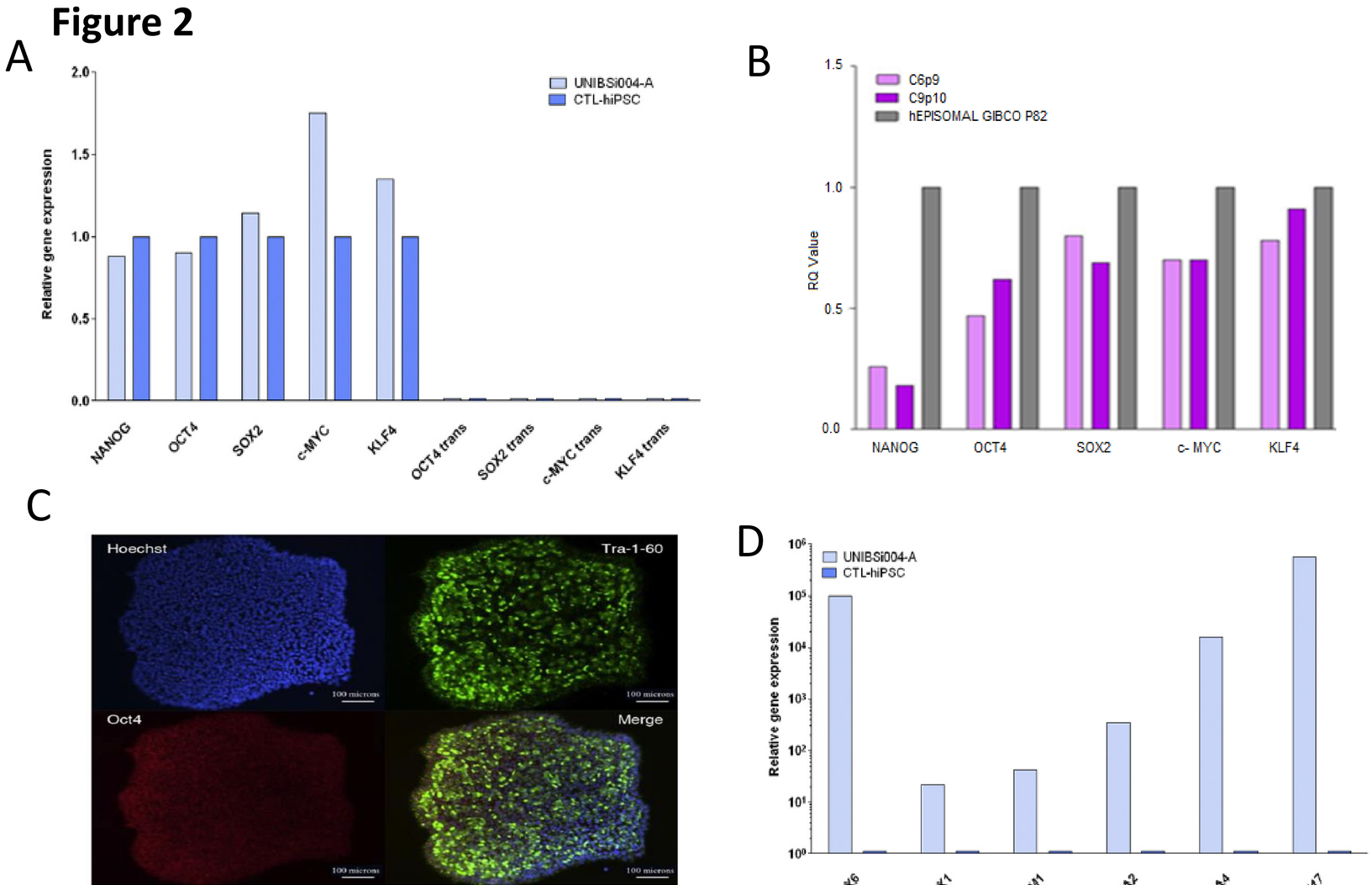

FIGURE 2 . Charactrization of CdC IPSCs patient (A &B) expression of endogenous NANOG, OCT4, SOX2, c-MYC, and KLF4 fully comparable commercial control iPSC line (CTL-hiPSC, Gibco® Episomal hiPSC Line **)** the absence of transgene expression confirmed the clearance of the viral vectors **(C)** Tra-1-60 marker is properly present on cell surface, while the transcriptional factor OCT4 is expressed at nuclear level **(D) UNIBSi004-A** IPSCs line at passage 33 was investigated for spontaneous differentiation capacity in the three germ layers by the expression of ectodermal, mesodermal and endodermal markers (PAX6-SOX1, NCAM1-ACTA2, GATA4-SOX17)

Q PCR for CTNND2, SEMA5A and hTETRT genes

Figure 3

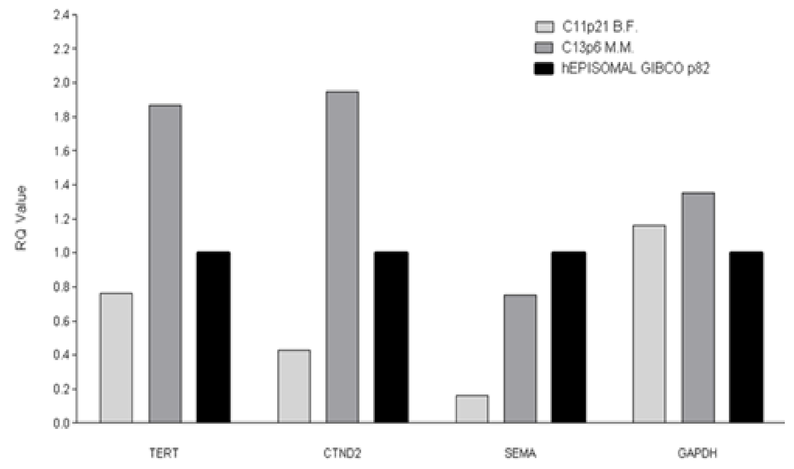

FIGURE 3.mRNA expression of the deleted genes In this image, the TERT, CTNND2 and SEMA5A gene expressions of the CdC-IPSC (C11P21BF), Control IPSC(C13p6M.M, HepisomalGibco p82)samples are represented.. TERT gene expression in CdC IPSC is half as compared to that of controls; CDNND2 value is about a fifth, and SEMA5A gene expression is about a quarter in the CdC IPSC as compared to the controls

Conclusions & future prospectives

➢These present preliminary results represent a fundamental starting point based, for the first time, on reprogrammed to pluripotency peripheral blood mononuclear cells derived from CdC patients

➢CdC-derived induced pluripotent stem cells (IPSCs) provide invaluable access to study the disease in differentiated cells derived from CdC iPSC such as neurons and Mesenchymal stem cells (MSCs).

➢Our studies will be oriented to understand the role of the deleted genes to fill this gap in the knowledge and shed light into disease processes involved in the CdC pathology.

➢We expect to identify distinctive molecular signatures with prognostic/diagnostic relevance that will have a significant impact in the knowledge of physiopathology, contributing to CdC diagnosis .

➢The different integrated approaches will constitute a gain for basic research and for further clinical studies with the aim to identify therapeutic agents with potential clinical benefit.

References

AjitkumCerruti Mainardi, P., 2006. Cri du Chat Syndrome. Orphanet J. Rare Dis. 1, 33.
ar, A., Whitten, R.A., Feb 26, 2018. Cri du Chat Syndrome. StatPearls.
Cornish, K.M., Pigram, J., 1996. Developmental and behavioural characteristics of Cri duChat Syndrome. Arch. Dis. Child. 75, 448–450.
Nguyen, J.M., et al., September, 2015. 5p Deletions: current knowledge and future directions.
Am. J. Med. Genet. C: Semin. Med. Genet. 169 (3), 224–238.