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Lab Resource: Stem Cell Line

Generation of KCL028 research grade human embryonic stem cell line carrying a mutation in the *HTT* gene

Laureen Jacquet [a], Heema Hewitson [a], Victoria Wood [a], Neli Kadeva [a], Glenda Cornwell [a], Stefano Codognotto [a], Carl Hobbs [b], Emma Stephenson [a], Dusko Ilic [a]

[a] Stem Cell Laboratories, Division of Women's Health, Faculty of Life Sciences and Medicine, King's College London and Assisted Conception Unit, Guy's Hospital, London, United Kingdom
[b] Histology Laboratory, Wolfson Centre for Age-Related Diseases, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom

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ABSTRACT

The KCL028 human embryonic stem cell line was derived from an embryo donated for research that carried an autosomal dominant mutation affecting one allele of the *HTT* gene encoding huntingtin (43 trinucleotide repeats; 21 for the normal allele). The ICM was isolated using laser microsurgery and plated on γ-irradiated human foreskin fibroblasts. Both the derivation and cell line propagation were performed in an animal product-free environment. Pluripotent state and differentiation potential were confirmed by in vitro and in vivo assays.

Resource table

Name of stem cell line	KCL028
Institution	King's College London, London UK
Derivation team	Neli Kadeva, Victoria Wood, Glenda Cornwell, Stefano Codognotto, Emma Stephenson
Contact person and email	Dusko Ilic, email: dusko.ilic@kcl.ac.uk
Date archived/stock date	May 25, 2011
Type of resource	Biological reagent: cell line
Sub-type	Human pluripotent stem cell line
Origin	Human embryo
Key marker expression	Pluripotent stem cell markers: NANOG, OCT4, TRA-1-60, TRA-1-81, alkaline phosphatase (AP) activity
Authentication	Identity and purity of line confirmed
Link to related literature (direct URL links and full references)	1) Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. Cytotherapy. 14 (1), 122–128. doi: 10.3109/14653249.2011.623692 http://www.ncbi.nlm.nih.gov/pubmed/22029654 2) Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. Nat. Protoc. 7 (7), 1366–1381. doi: 10.1038/nprot.2012.080 http://www.ncbi.nlm.nih.gov/pubmed/22722371

(*continued*)

Name of stem cell line	KCL028
	3) Jacquet, L., Neueder, A., Földes, G., Karagiannis, P., Hobbs, C., Jolinon, N., Mioulane, M., Sakai, T., Harding, S.E., Ilic, D., 2015. Three Huntington's disease specific mutation-carrying human embryonic stem cell lines have stable number of CAG repeats upon in vitro differentiation into cardiomyocytes. PLoS One. 10(5), e0126860. http://doi.org/10.1371/journal.pone.0126860 http://www.ncbi.nlm.nih.gov/pubmed/25993131
Information in public databases	KCL028 is a National Institutes of Health (NIH) registered hESC line NIH registration number: 0224 NIH approval number: NIHhESC-13-0224 http://grants.nih.gov/stem_cells/registry/current.htm?id=664
Ethics	The hESC line KCL028 is derived under license from the UK Human Fertilisation and Embryology Authority (research license numbers: R0075 and R0133) and also has local ethical approval (UK National Health Service Research Ethics Committee Reference: 06/Q0702/90). Informed consent was obtained from all subjects and the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the NIH Belmont Report. No financial inducements are offered for donation.

Resource details

Consent signed	Jan 27, 2011
Embryo used	May 04, 2011
UK Stem Cell Bank deposit approval	Dec 01, 2011 Reference: SCSC11-47

http://dx.doi.org/10.1016/j.scr.2016.01.006

(continued)

Sex	Male 46, XY
Grade	Research
Disease status (Fig. 1)	Mutation affecting one allele of the *HTT* gene encoding huntingtin (~43 CAG repeats; 21 for the normal allele) associated with Huntington's disease (Ilic et al., 2015)
Karyotype (aCGH)	No imbalance detected.
DNA fingerprint	Allele sizes (in bp) of 17 microsatellite markers specific for chromosomes 13, 18 and 21 (Jacquet et al., 2015)
HLA typing	HLA-1: 02,03; −B:07,35, −C: 04,07; Bw: 6; DRB1: 01; DQB1: 05
Viability testing	Pass
Pluripotent markers (immunostaining) (Fig. 2)	NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity (Jacquet et al., 2015)
Three germ layers differentiation in vitro (immunostaining) (Fig. 3)	Endoderm: AFP (α-fetoprotein); Ectoderm: TUBB3 (tubulin, β3 class III); Mesoderm: ACTA2 (actin, α2, smooth muscle) (Jacquet et al., 2015)
Three germ layer differentiation in vivo (teratomas) (Fig. 4)	Endoderm: AFP, GATA4. Ectoderm: TUBB3, GFAP (glial fibrillary acidic protein). Mesoderm: DES (desmin), Alcian Blue and periodic acid–Schiff (PAS)-stained cartilage (Jacquet et al., 2015)
Targeted differentiation	Cardiomyocytes: TNNT2 (cardiac troponin T) immunostaining
Sibling lines available	KCL027

We generated KCL028 research grade hESC line following protocols, established previously (Ilic et al., 2012; Stephenson et al., 2012). The expression of the pluripotency markers was tested after freeze/thaw cycle (Ilic et al., 2012). Differentiation potential into three germ layers was verified in vitro (Jacquet et al., 2015) and in vivo (Jacquet et al., 2015).

Materials and methods

Consenting process

We distribute Patient Information Sheet (PIS) and consent form to the in vitro fertilization (IVF) patients if they opted to donate to research embryos that were stored for 5 or 10 years. They mail signed consent back to us and that might be months after the PIS and consent were mailed to them. If in the meantime new versions of PIS/consent are implemented, we do not send these to the patients or ask them to re-sign; the whole process is done with the version that was given them initially. The PIS/consent documents (PGD-V.8) were created on Jul. 01, 2010. HFEA Code of Practice that was in effect at the time of document creation: Edition 8 – R.2 (http://www.hfea.gov.uk/2999.html). The donor couple signed the consent on Jan. 12, 2011. HFEA Code of Practice that was in effect at the time of donor signature: Edition 8 – R.2. HFEA Code of Practice Edition 8 – R.2 was in effect: Apr. 07, 2010–Apr. 06, 2011.

Embryo culture and micromanipulation

Embryo culture and laser-assisted dissection of inner cell mass (ICM) were carried out as previously described in details (Ilic et al., 2012; Stephenson et al., 2012). The cellular area containing the ICM was then washed and transferred to plates containing mitotically inactivated human neonatal foreskin fibroblasts (HFF).

Cell culture

ICM plated on mitotically inactivated HFF were cultured as described (Ilic et al., 2012; Stephenson et al., 2012). TE cells were removed mechanically from outgrowth (Ilic et al., 2007; Ilic et al., 2010). hESC colonies were expanded and cryopreserved at the third passage.

Viability test

Straws with the earliest frozen passage (p. 2–3) are thawed and new colonies are counted three days later. These colonies are then expanded up to passage 8, at which point cells were part frozen and part subjected to standard battery of tests (pluripotency markers, in vitro and in vivo differentiation capability, genetics, sterility, mycoplasma).

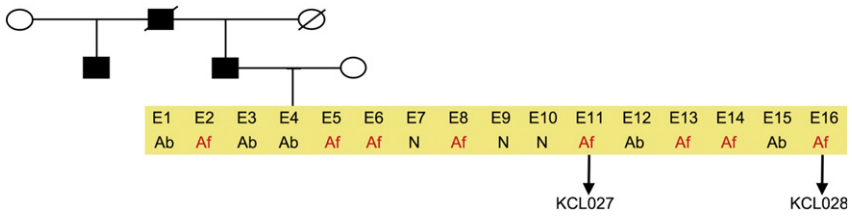

Fig. 1. Genetic pedigree tree. The couple undergoing IVF had 16 embryos in this particular cycle. Three embryos were normal, whereas those that carried the mutation in *HTT* were donated for research. We derived hESC lines from two of them.

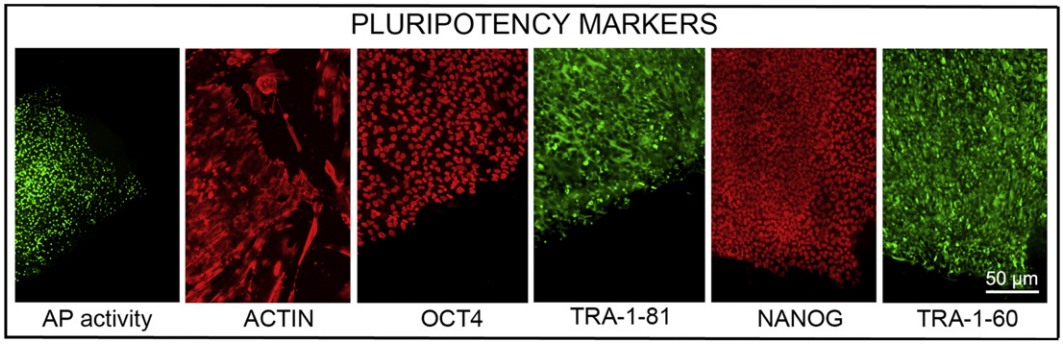

Fig. 2. Expression of pluripotency markers. Pluripotency is confirmed by immunostaining (Oct. 4, Nanog, TRA-1-60, TRA-1-81) and alkaline phosphatase (AP) activity assay. Actin stress fibers, visualized with rhodamine-phalloidin (red), are present in both feeders and hES cell colonies, whereas AP activity (green) is detected only in hES cells. Scale bar, 50 µm.