Human lymphoblast cells from a female and male patient diagnosed with Alzheimer’s disease (AD) with different genotypes of a functional copy number variation (CNV) in the AD risk gene CR1 were used to generate integration-free induced pluripotent stem cells (iPSCs) employing episomal plasmids expressing OCT4, SOX2, NANOG, LIN28, c-MYC and L-MYC. The iPSCs retained the CR1 CNV, and comparative transcriptome analyses with the human embryonic stem cell line H1 revealed a Pearson correlation of 0.956 for AD1-CR10 and 0.908 for AD1-CR14.

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were reprogrammed employing OriP/EBNA-1-based episomal plasmids expressing OCT4, SOX2, KLF4, C-MYC, L-MYC, LIN28 and a p53 shRNA. After picking the clones, both clones of each iPSC lines, namely AD1-CR10 and AD1-CR14, retained traces of EBNA-1 (Fig. 1A) but was diluted out after several passages \( \text{data not shown} \). Pluripotency was confirmed by (i) expression of OCT4, SOX2, NANOG, TRA-1–60 and TRA-1–81 and SSEA4 (Fig. 1B) and (ii) embryoid body (EB)-based spontaneous differentiation into cell types representative of the three germ layers, namely ectoderm (Nestin, PAX6), mesoderm (SMα – smooth muscle actin) and endoderm (AFP – α-Feto Protein, SOX17) (Fig. 1C).

The DNA fingerprint of AD1-CR10 iPSC line was identical to the parental lymphoblast line Lymph10, whereas iPSC line AD1-CR14 was identical to lymphoblast cells Lymph14 (Fig.1D). Karyotype analysis exhibited a normal diploid chromosomal content for all four lines and was female (XX) for Lymph14 and AD2-CR14 lines, and male (XY) for Lymph10 and AD2-CR10 lines (Fig. 1E). As depicted in the Dendrogram (Fig. 1F), the transcriptome of the iPSC lines, AD1-CR10 and AD1-CR14, and the embryonic stem cell line H1 clustered together with a Pearson correlation of 0.908 (AD1-CR14) and 0.956 (AD1-CR10). The reprogramming process did not alter the CR1 CNV class as demonstrated in Fig. 1G.

2. Materials and methods

2.1. Ethic statements

The EBV-transformed lymphoblastoid cell lines, Lymph10 and Lymph14, used for the generation of the iPSC lines AD1-CR10 and AD1-CR14, was generated from peripheral blood lymphocytes from a male 78- and a female 66-year-old donor diagnosed with Alzheimer’s disease. The research protocol was approved by the Ethics Committee of the University Hospital Antwerp and the University of Antwerp, Belgium.

2.2. Cell culture

The lymphoblast cell lines Lymph10 and Lymph14 (Brouwers et al., 2012) were cultured in RPMI1640 supplemented with 15% fetal bovine serum (Invitrogen™), 1% Glutamax (Invitrogen™), 1% Sodium pyruvate (Invitrogen™) and 1% Penicillin/Streptomycin (Invitrogen™) at 37 °C and 5% CO2.

2.3. Derivation of the iPSC cell line

Lymphoblast cells (Lymph10, Lymph14) were reprogrammed by nucleofection of OriP/EBNA-1-based episomal plasmids (expressing OCT4, SOX2, KLF4, L-MYC, LIN28 and a p53 shRNA) (Okita et al., 2011) at the Biomedicum Stem Cell Center, University of Helsinki, Finland, \( \text{http://research.med.helsinki.fi/neuro/Otonkoski/core/default.html} \) as a service. The iPSC cells were cultured under feeder-free conditions on Matrigel®-coated plates in E8 medium (Invitrogen™).

2.4. Polymerase chain reaction

RT-PCR to assess the expression levels of the transgene and endogenous stem cell markers were carried out by the Biomedicum Stem Cell Center, University of Helsinki, Finland as described in (Schröter et al., 2016a; Schröter et al., 2016b).

2.5. Embryoid body formation

Embryoid body (EB) formation was carried out as described in (Schröter et al., 2016a; Schröter et al., 2016b). In brief, after culturing of the iPS cells in ultra-low attachment flask (Corning) in FDTA medium, the EBs were replated onto gelatin-coated plates, again in FDTA medium lacking bFGF and Dorsomorphin (Frank et al., 2012).

2.6. Immunocytochemistry

Immunocytochemistry was performed as described in (Schröter et al., 2016a; Schröter et al., 2016b), except for the germ layers staining. The following antibodies were used: rabbit anti-Nestin (Sigma Aldrich; 1:1000), mouse anti-PAX6 (SYSV, 1:1000), mouse anti-SMA (Cell Signaling; 1:1000), and mouse anti-SOX17 (Novus Biological, 1:500). The fluorescent images obtained by an inverse fluorescence microscope LSM 700 (Carl Zeiss) and analyzed employing Adobe Photoshop software (Adobe, USA).

2.7. Karyotype analysis

Karyotype analysis was evaluated and performed at the Institute of Human Genetics and Anthropology, Heinrich-Heine-University, Düsseldorf. Twenty-six metaphases for the parental lymphoblast line Lymph10 and twenty-five metaphases for the iPSC line AD2-CR10 as well as twenty metaphases for the parental lymphoblast line Lymph14 and fifteen metaphases for iPSC line AD2-CR14 were counted, respectively.

2.8. DNA fingerprinting

Genomic DNA was isolated according to the manufacturer protocol from the lymphoblast line AD2-2, the Lymph2-AD2-iPS line, separated in two independent lines A and B, and the human embryonic stem cell line (H1). The STR analysis was performed by PCR amplification with specific primers (Prigione et al., 2011; Wang and Adjaye, 2011).

2.9. Dosage analyses

Copy number status of the CR1 CNV was determined using multiplex amplicon quantification (MAQ; Multiplicom N.V., Niel, Belgium) as described in (Brouwers et al., 2012). Briefly, three amplicons in the CR1 locus, targeting three LCRs, and six control amplicons outside the CR1 locus were amplified in one multiplex PCR reaction and resolved on an Applied Biosystem 3730X/DNA analyzer (Applied Biosystem, Foster City, CA, USA). Dosage quotients were calculated on normalized peak areas using MAQ-S software. Copy number of amplicon CR1 01, targeting the first LCR in CR1, determines CR1-F and -S isoform.

2.10. RNA-based microarray analysis

RNA-based microarray analysis was performed as described in (Schröter et al., 2016a; Schröter et al., 2016b). Microarray analysis was outsourced to the Genomics/Transcriptomic Laboratory of the BMFZ, Heinrich-Heine-University, Düsseldorf.

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Fig. 1. Characterization of the iPS lines AD1-CR10 and AD1-CR14. (A) Confirmation of mRNA expression of OngIP and EBNA-1 genes; NTC: non-template control; PosCo: control line with OriP and EBNA-1 detection. (B) Immunofluorescence-based detection of human pluripotency-associated proteins OCT4, SOX2, c-MYC, NANOG and surface markers TRA-1-60 and TRA-1-81. Hoechst 33,258 was used for the nuclei staining. Scale bar: big – 100 μm, small – 50 μm. (C) Embryoid body (EB) formation was induced in AD1-CR10 and AD1-CR14 iPSCs in vitro and analyzed by immunofluorescence-based detection of different germ layer marker: ectoderm – Nestin, PAHX; mesoderm – α-smooth muscle actin (SMA) and endoderm – SOX17. Scale bar: 25 μm. (D) Gel electrophoresis of DNA fingerprinting PCR products. Genomic DNA was isolated from parental lymphoblast cells Lymph10 and Lymph14, AD1-CR10 and AD1-CR14 iPSCs and embryonic stem cell line H1. DNA was amplified using PCR primers that flank different genomic regions (D10S1214 and D21S2055). (E) Karyotyping analysis of parental lymphoblast Lymph10 and AD1-CR10-iPSC line, presence of male karyogram 46, XY, and Lymph14 and AD1-CR14-iPSC line, presence of female karyogram 46, XX. (F) Cluster Dendrogram of AD1-CR10 and embryonic stem cell line H1. (G) Dosage plot of the iPS lines AD1-CR10 (black symbols) and AD1-CR14 (gold symbols) for six control amplicons and three amplicons in CR1, the black triangle indicates the ampiclon determining CR1 CNV class. The y-axis presents dosage quotients (DQ) calculated using four references. DQ-values within the grey zone indicate normal copy number. The dosage plot of iPSCs AD1-CR10 is compatible with CR1-F/F; the dosage plot of iPSCs AD1-CR14 shows a heterozygous duplication of the first LCR, hence is compatible with CR1-F/S. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)