Final report on the research project: "Modelling of DiGeorge syndrome by pluripotent stem cells"

I. Background, scientific questions of the project

Understanding disease mechanisms is a prerequisite for developing tailored therapies, while in many cases; no proper animal or in vitro models are available. In disorders with complex developmental and functional alterations, the use of induced pluripotent stem cells (iPSC) may promote a better understanding of the underlying mechanisms. Stem cell-based platforms are widely used to study monogenic disorders. These models describe particular disease-related phenotypes and can also be applied to test cellular responsiveness to clinically applicable drugs in cell cultures. However, a major hurdle is studying complex diseases where phenotypes are affected by multiple genes, environmental interactions, involvement of more than one organ, or dysfunctional inaccessible cell types. Our project aims to investigate whether this approach may be suitable for understanding complex disorders such as DiGeorge syndrome.

The DiGeorge syndrome is a microdeletion syndrome; deletion of multiple genes leads, amongst others, to congenital heart disease and neuronal disorders. These conditions may also appear as *in vitro* phenotypes. Therefore, in this project, our specific aims were:

1, to generate hiPSCs from blood cells of patients suffering from the DiGeorge syndrome (as well as those of healthy relatives as controls).

2, to determine whether the differences in the in vitro phenotypic profiles of the diseased and control hiPSCs, progenitors and differentiated tissues reflect the alterations in the patients.

3, and evaluate how the lack of DGCR8, one of the critical genes deleted in this syndrome, modulates cell differentiation and microRNA signalling that may be involved in cell differentiation alterations observed in the DiGeorge syndrome.

The obtained differences will lead us to understand better the underlying disease mechanisms, which is of utmost importance for the future development of diagnostic tools and therapeutic approaches.

<u>The 22q11.2 deletion syndrome:</u> The 22q11.2DS (also called Velocardiofacial syndrome, or DiGeorge syndrome) is a rare disease, although the most common chromosomal microdeletion disorder (prevalence is approximately 1 per 1000 foetuses (Dar, Jacobsson et al. 2022). The microdeletion causes haploinsufficiency with various phenotypic manifestations, and the most typical symptoms are 1. immunodeficiency due to the absence of thymus, 2. hypocalcaemia as a consequence of hypoplasia of the parathyroid gland, 3. congenital cardiovascular anomalies, 4. neuropsychiatric disorders (ADHD in childhood and schizophrenia in adults). Several other symptoms, such as renal, ocular, and skeletal malformations, hearing loss, and laryngeal abnormalities, may also occur. The mostly *de novo* occurring deletion results in a 1.5–3 Mb microdeletion on the long arm of chromosome 22 (~90% of patients have a common ~3 Mb deletion). However, the deletion can also be inherited, and familial autosomal dominant recurrence have been reported in ~10% of

patients. Interestingly, the highly variable phenotypic expression and the severity of symptoms are not correlated with the size of the deletion, and disease severity usually increases when inherited (Cirillo, Giardino et al. 2014). The microdeletions may involve 50 protein-coding genes in this genetic region, but 90 genes can be affected in the disease altogether. Among these, the DGCR8 gene is of particular interest, because this is a subunit of the microprocessor complex, which has an important role in miRNA maturation, and miRNAs are associated with several developmental processes (McDonald-McGinn, Sullivan et al. 2015).

II. Key results of the project

1, Selection of the family with 3 generation pedigree of the DiGeorge syndrome

As inherited and familial autosomal dominant recurrence is relatively low (~10% of patients), families with three generations of DiGeorge syndrome are extremely rare. Only two families have been reported till now (Iascone, Vittorini et al. 2002, Vergaelen, Swillen et al. 2015), and no iPSC lines were established from them. We checked the Hungarian registry and selected a family with DiGeorge syndrome for further investigation.

2, Generation of iPSC lines from DiGeorge patients

In the family involved in our study, we have three patients manifesting the disease with different severity and two healthy relatives (Fig. 1). Grandfather showed mild symptoms, articulation disorder and minimal facial dysmorphia. Moderate symptoms of the mother involved vascular ring (surgically corrected in childhood), hypocalcaemia and minimal facial dysmorphia. Symptoms of the child were severe and led to death at age 5 months; tetralogy of Fallot (ventricular septal defect, pulmonary atresia, right ventricular hypertrophy, misplaced aorta), atrial septal defect, asymmetrical brain ventricles, hypocalcaemia, hypoparathyroidism, minimal facial dysmorphia.

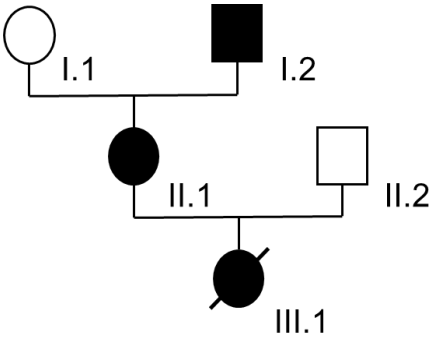


Figure 1. Pedigree of the family involved in the study

According to the permission for reprogramming and the study by the Human Reproduction Committee of the Hungarian Heath Science Council (ETT HRB- Approval number: 42592-2/2016-EHR), blood samples were obtained from patients and healthy relatives after written informed consent. Blood mononuclear cells were isolated from patients (grandfather, mother and daughter diagnosed with DiGeorge syndrome) as well as healthy relatives (grandmother and father) and were reprogrammed into induced Pluripotent Stem Cell (iPSC) lines by commercially available Sendai virus kit. Three individual clones (for each sample) were

picked and cultured separately thereafter. Next, Mycoplasma-free culture conditions and karyotype were tested after 15 passages.

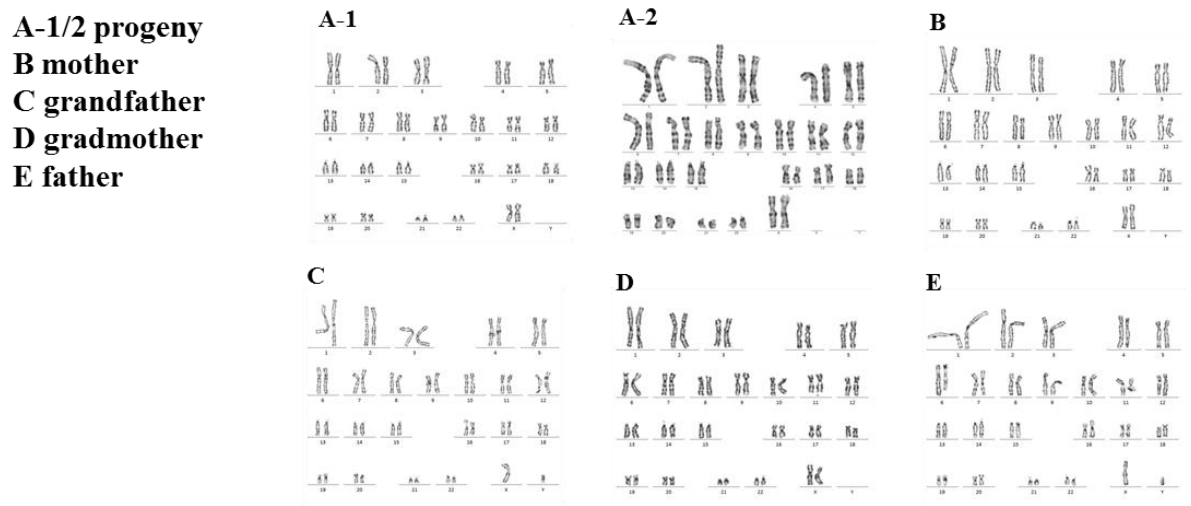


Figure 2. Karyotype analysis of resulted iPSC clones

We have found clones with normal karyotype for every member of the family (2 clones from the daughter and 1-1 from the other relatives) except the father. As we found a translocation between 6 and 12 chromosomes in the father's derived samples (blood and iPSCs as well), we included a commercially available iPSC line (XCl-1, umbilical cord blood-derived iPSC line) as another control cell line (and age-matched control to the daughter) into the study. The identities of the clones were verified by STR analysis of iPSCs and iPSC-matched blood samples (data not shown).

We have proven sustained microdeletions in patient-derived lines by comparing blood samples to iPSC lines using MLPA and found the same microdeletion in all patients' samples (grandfather, mother and daughter). Moreover, hiPSCs of DiGeorge patients showed significantly lower mRNA expression of DGCR8 gene than controls (data not shown), consistent with the genetic background.

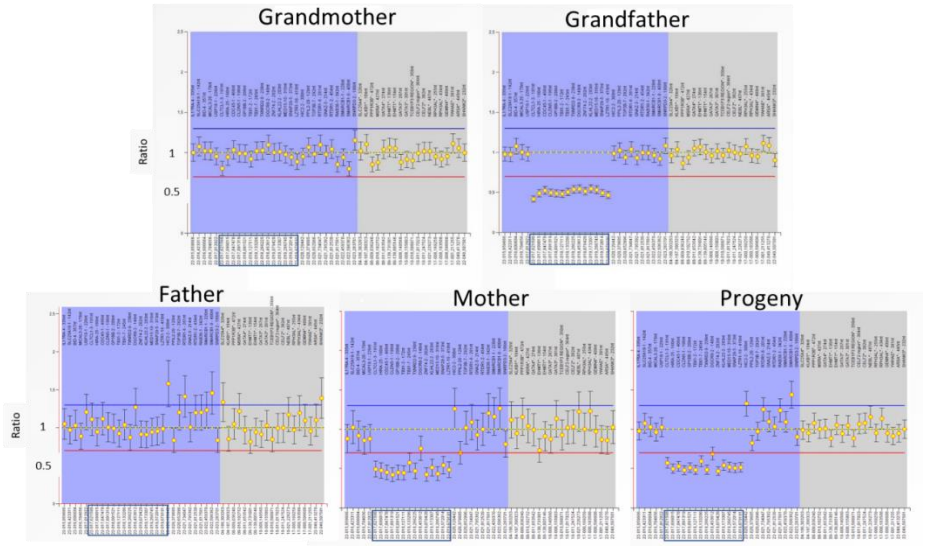
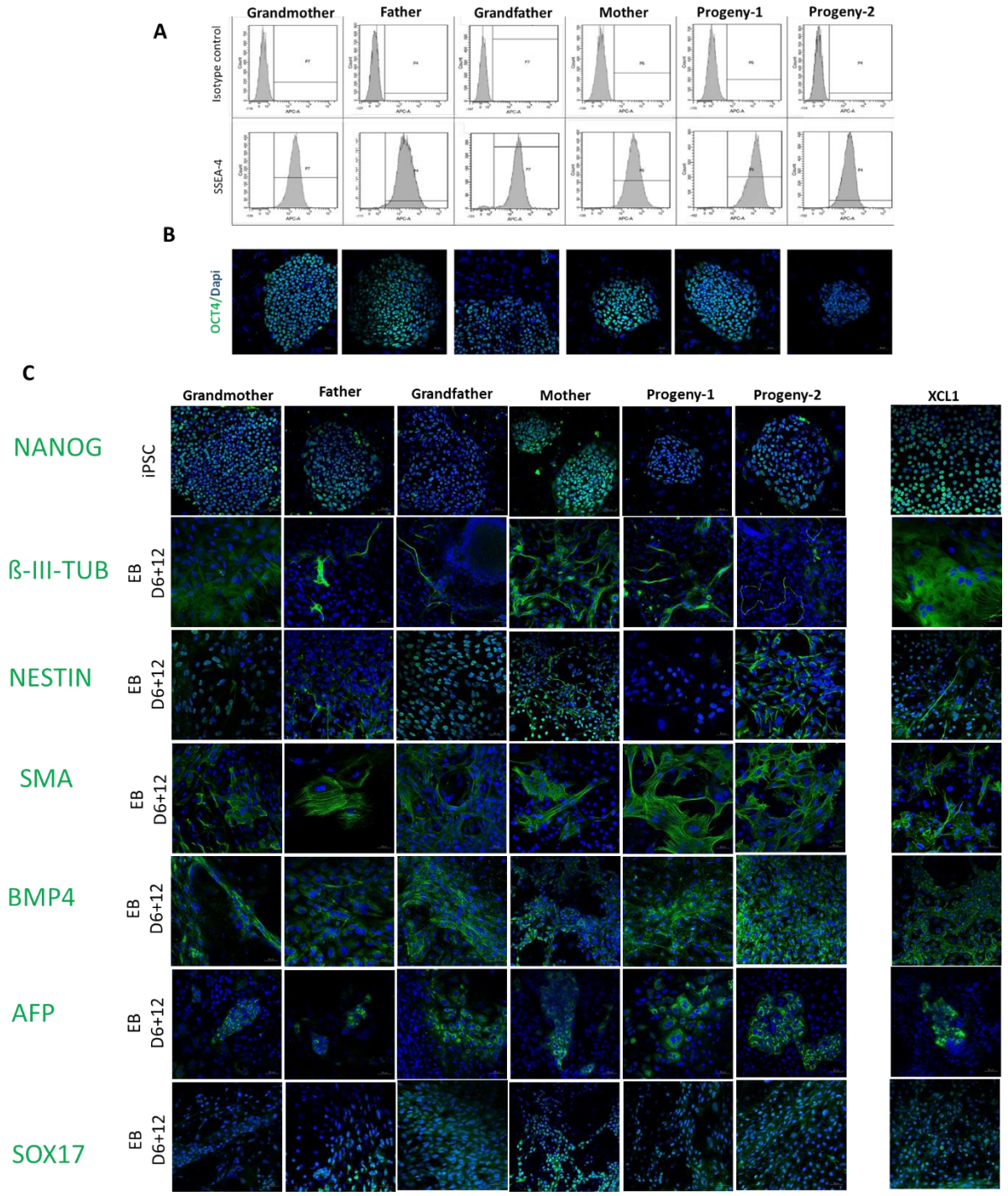


Figure 3. Typical 3 Mb long monoallelic deletion in hiPSCs of DiGeorge patients shown by MLPA

Standard pluripotency assays were performed on clones with normal karyotype (2 clones from the daughter and 1-1 from the other relatives; see figure 2). These comprised testing mRNA and protein levels of pluripotency markers OCT4, Nanog, and SSEA4 (fig.4). By using a 3D EB culture method, we verified that pluripotent clones can give rise to all the three germ layers by assessing germ layer markers (AFP and SOX17-for endoderm, BMP4 and SMA-for mesoderm, Nestin and B-III-tubulin for ectoderm, and parallel silencing of pluripotency genes Oct4 and Nanog).



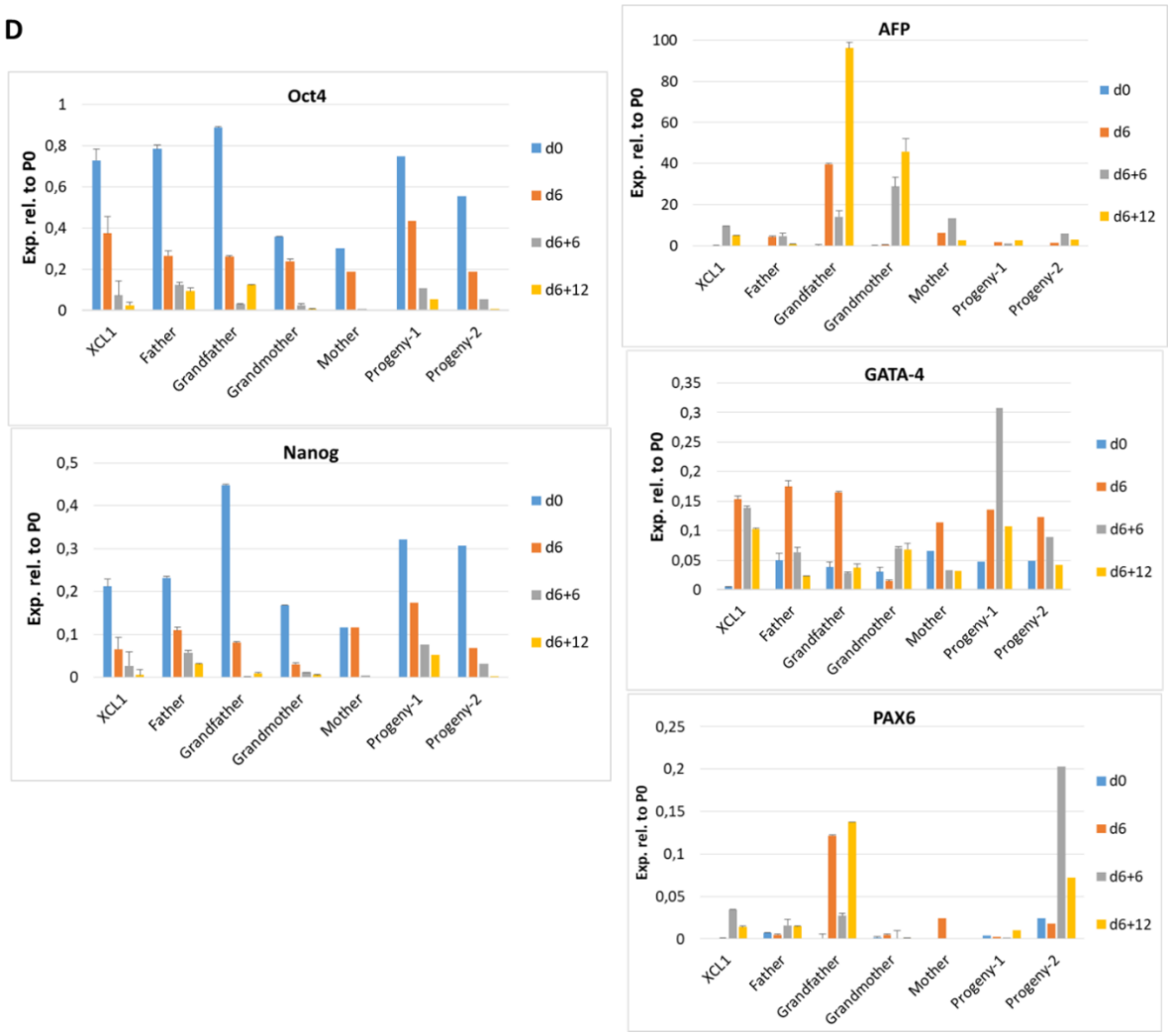


Figure 4. Generation of iPSC from PBMCs of donors A) SSEA pluripotency marker expression of the established clones measured by FACS B) Immunostaining for OCT4 pluripotency marker of the established clones C-D) hiPSC differentiated by EB method express germ layer markers while downregulate Oct4 and Nanog pluripotency markers shown by ICC (C) and qPCR (D).

3, Cardiac differentiation and characterisation of cardiomyocytes

Next, hiPSCs were differentiated into cardiomyocytes based on a previously described protocol (Burridge, Holmstrom et al. 2015). Cardiomyocytes started beating between days 7 and 15 of cardiac differentiation. Cardiac cell cultures of the mother and child showed morphological differences in structure compared to controls and grandfather (Fig. 5. A). Detachment of cardiac clusters from the surface was observed in the case of both mother and child. In contrast, CMs of the mother often formed globular structures, and fibre-like morphology was frequently observed in CM cultures of the child. Control CMs and those of the grandfather stayed attached in cardiac monolayers or sheets of cells.

Despite the initial differences, passaging and plating cardiomyocytes with the same cell density, cardiac cells from all cell lines assembled and formed syncytium. Immunostaining with the cardiac-specific marker TNNI3 showed high purity of cardiomyocytes in the cell culture (> 95%) (Fig. 5. B). This was further confirmed by mRNA expression analysis of multiple cardiac differentiation markers, with no difference between cell lines (Fig. 5. C.).

The used differentiation protocol yields a mixed population of cardiac subtypes, and cells thus express ventricular (MYL2), atrial (MYL7) and pacemaker (HCN4) markers (Lee, Protze et al. 2017).

Cardiac gap junction proteins (connexins) have been implicated to have a role in the development of cardiac anomalies, including TOF (Salameh, Blanke et al. 2013). Cx40 (GJA5) and Cx43 (GJA1) are two major gap junction proteins in the heart. Measurement of the expression of these two genes in cardiomyocytes showed a significantly lower level of GJA1 in the child's cells. In contrast, no difference was observed in the expression of GJA5 (Fig. 5. D).

The DiGeorge-associated gene DGCR8 in CMs of DiGeorge patients showed significantly lower levels than in controls. However, in comparison with the controls, TBX1 expression in the DiGeorge group was not statistically significant (Fig. 5. D).

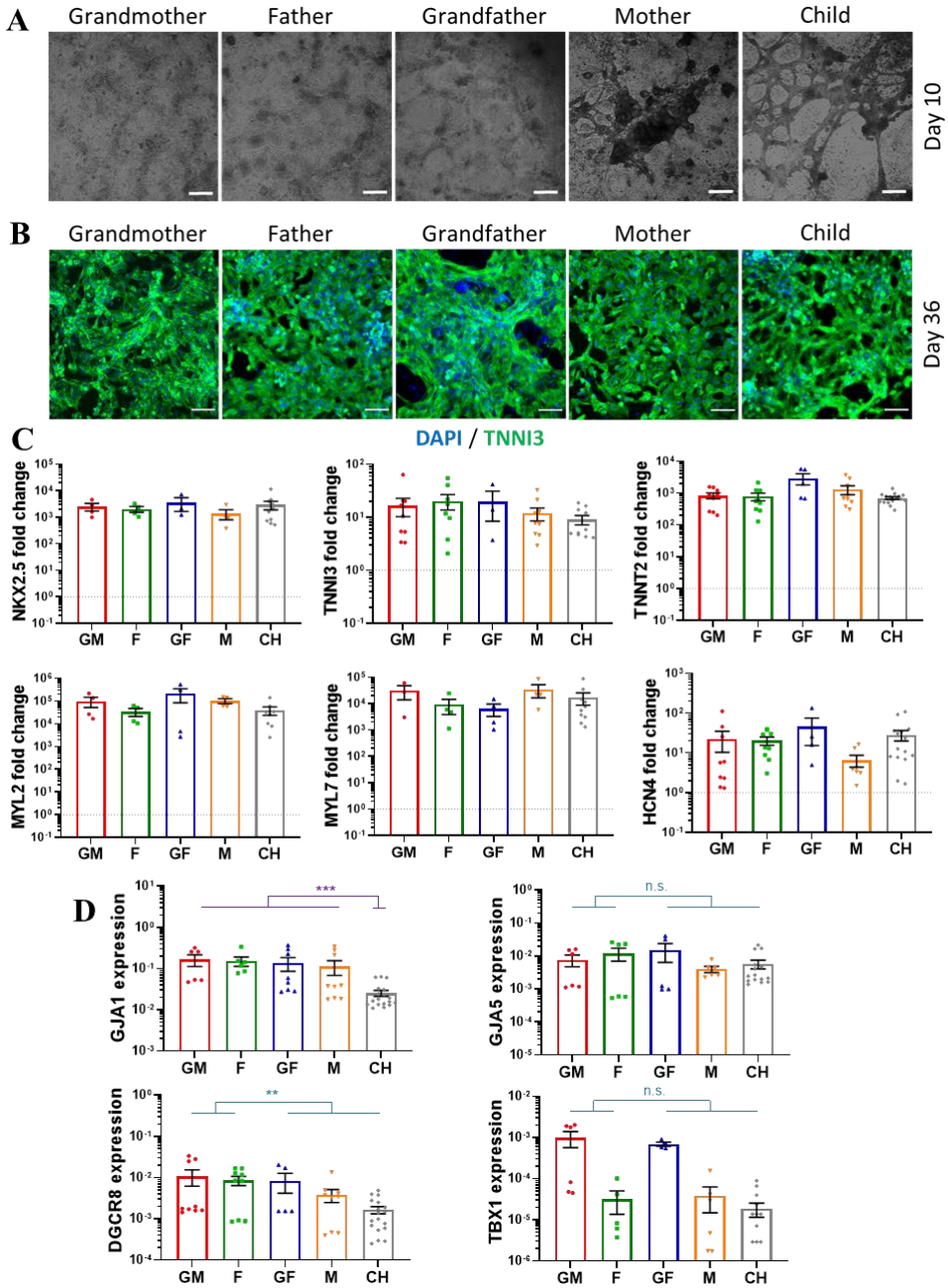


Figure 5. Characterisation of hiPSC-derived cardiomyocytes
*(**A**) Transverse light microscopic images of hiPSC-derived cardiomyocytes on day 10 of cardiac differentiation (scale bar: 500 mm). (**B**) Immunostaining for cardiac marker TNNI3 on day 36 (scale bar: 100 mm). (**C**) mRNA expression measurements of cardiac differentiation markers on day 36, fold change compared to expression in respective hiPSCs. (**D**) mRNA levels of cardiac connexins and DiGeorge-associated genes. (GM: grandmother, F: father, GF: grandfather, M: mother, CH: child). Data come from at least 2 biological and 3-3 technical parallels.*

We have developed high-content immunocytochemistry assays to characterise cardiomyocyte structural properties of hiPSC-CMs in culture. Cells were scanned on a confocal Opera Phenix Plus plate reader (PerkinElmer). Cells displayed structural features of the immature phenotype in terms of shape and sarcomeric pattern, and different sarcomere organisation. Cell morphology has been evaluated for sarcomere reorganisation (entropy as an endpoint), cell size, and cell number (reflecting cell death and proliferation balance) (Fig.6.). Cell area was modestly higher in DiGeorge-derived cells ($p<0.05$) than those in control relatives. Minor differences were detected also in sarcomere texture parameters, suggesting some variability in cardiomyocyte maturation and remodelling.

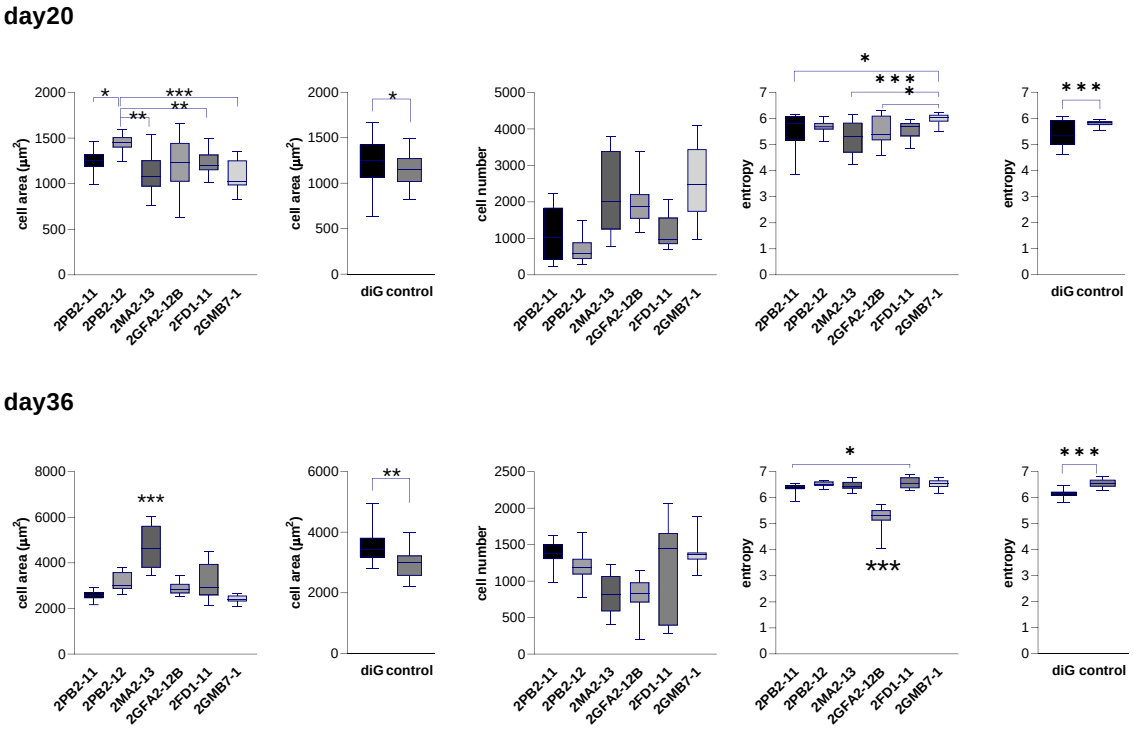


Figure 6.Morphological analysis of cardiomyocytes
Cell size, and cell number (reflecting cell death and proliferation balance), and sarcomere reorganisation (entropy as an endpoint) were analysed from 3 biological parallels.

For functional characterisation, we have developed an optical-flow-based method to quantify the dynamic behaviour of human pluripotent stem cell-derived cardiomyocytes (Izadifar, Berecz et al. 2022). Time-lapse images of iPSC-CMs were acquired using a high spatial and time-resolution phase-contrast video microscopy. Speckled images were characterised by evaluating the cross-correlation coefficient, speckle size, speckle contrast and speckle quality of the images. We analysed the dynamic behaviour of iPSC-CMs, such as contraction velocity

field, contraction-relaxation strain rate, beating rate and contractile cycles. The analyses demonstrated qualitative differences in the contractile cycle patterns of iPSC-CMs during the contraction-relaxation phases between the child most severely affected by DiGeorge syndrome and the other family members. The quantitative characterisation of the contractile cycles revealed no significant difference between the iPSC-CMs of the child and those from the other family members. In contrast, iPSC-CMs from the mother, who is also affected by DiGeorge syndrome, exhibited significantly lower contraction speed and strain rate than those from the father and grandmother, who are unaffected (manuscript is under preparation).

Analysis of intracellular calcium signals allows us to study calcium transient kinetics, depending on beating frequency. Interestingly, the DiGeorge patient group showed higher frequency and shorter times for most calcium kinetics parameters (Fig. 7. A-C). Next, to test if *in vitro* phenotypes of cardiomyocyte function can be associated with patients with cardiovascular symptoms, hiPSC-CMs of mother and child were compared to the group of individuals without cardiovascular symptoms (controls and grandfather). No significant differences in frequency and calcium transient kinetics were observed in this case, suggesting that altered cardiomyocyte function is not the cause of cardiovascular symptoms in DiGeorge syndrome (Fig.7. A-C).

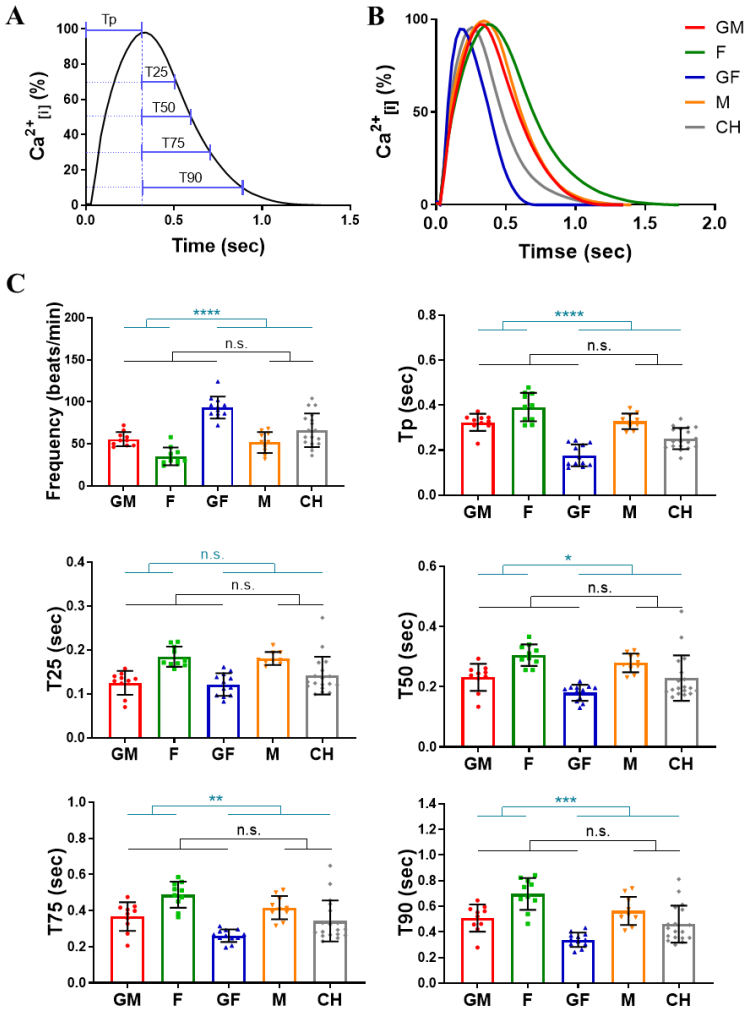


Figure 7. Analysis of calcium transient kinetics in hiPSC-derived cardiomyocytes

(A) Schematic representation of calcium kinetic parameters, Tp: time to peak, T25: 25 % decay, T50: 50 % decay, T75: 75 % decay, T90: 90 % decay. (B) Intracellular calcium changes measured on day 36 of cardiac differentiation; mean values are shown for each cell line. (C) Analysis of calcium kinetic parameters. (GM: grandmother, F: father, GF: grandfather, M: mother, CH: child). Data come from at least 2 biological and 3-3 technical parallels.

4, Endothelial differentiation

Endothelial cells represent another relevant cell type possibly contributing to the cardiovascular symptoms in DiGeorge syndrome. We optimised a previously described protocol for endothelial differentiation for each iPSC clone (Prasain, Lee et al. 2014). We generated iPSC-ECs, inducing differentiation with four factors: Activin-A, BMP4, bFGF and VEGF165. At day 12 of differentiation, the cultures were stained for CD31 endothelial marker (Figure 8 A) and sorted for CD31 positivity by FACS. Next we analysed the stained samples using ImageXpress Micro XLS high content screening device (Molecular Devices). Six fields of view (approximately half of the total well surface area) were imaged using a DAPI filter cube (ex. 377/50 nm, em. 447/60 nm), a FITC filter cube (ex. 482/35 nm, em. 536/40 nm) with a 10x Nikon objective (Plan Fluor, NA = 0.3). The result showed that the tube length and area did not vary among the samples, while tube thickness, branch points, segments and node area were significantly altered. It was more obvious when the patients with cardiovascular phenotype (mother and child) were compared to the samples with no cardiovascular symptoms (grandmother, grandfather and father).

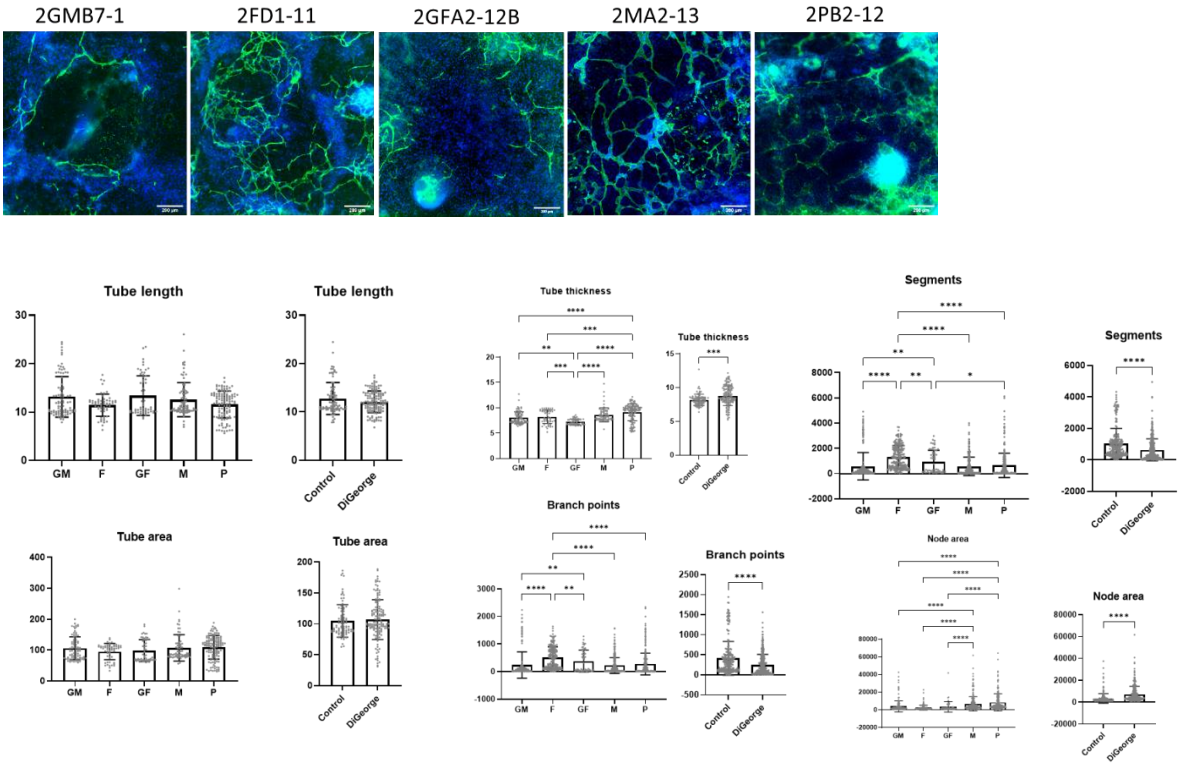


Figure 8. CD31 staining of the 12 days old endothelial cultures (upper panels) and morphological analysis of endothelial cultures (2 biological and 9-9 technical parallels - lower panels).

Sorted endothelial cells proliferate and can be further cultured. Cells were collected on day 19 of differentiation for molecular characterisation. mRNA expression levels relative to GAPDH housekeeping gene and fold change levels of endothelial cells relative to iPS cells were analysed. Levels of the pluripotency marker OCT4 decreased in endothelial cells compared to iPSCs. Endothelial markers PECAM-1 (CD31) and VE-cadherin (CD144) were elevated in endothelial cells and we experienced significant increase in expression levels of the child's cells compared to cells of the two controls and to mother in both cases. Fold change of CD31 in child's cells was significantly higher in comparison with grandmother and grandfather, and fold change of VE-cadherin was significantly higher compared to the two controls and to grandfather. We also investigated VEGF-A, a major angiogenic factor acting on endothelial cells, expression in endothelial cell cultures.

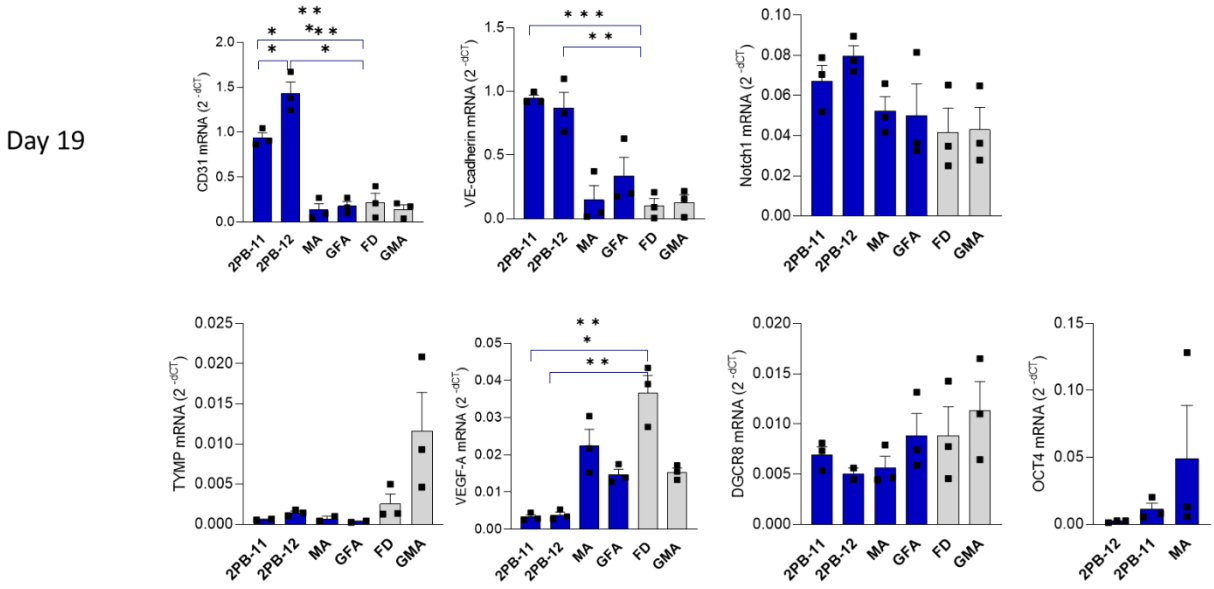


Figure 9. mRNA expression measurements of endothelial differentiation markers on day 19, fold change compared to expression of GAPDH.

Because we found significant differences in the expression of endothelial markers, we collected samples during endothelial differentiation to explore the underlying mechanisms. The samples were prepared for RNA sequencing on days 0, 5, 12 and 19, and the analysis was made in the framework of an international collaboration (with Laura Keruso's lab- NIH Washington). We have the raw data; further analysis will be carried out in the coming weeks.

5, Genetic background

We hypothesise that the observed phenotype may be caused by mutations in the DiGeorge region and/or the rest of the genome. Therefore, we performed whole exome sequencing to evaluate mutations in our iPSC lines. Genomic DNA from iPS cell lines was isolated using the NucleoSpin DNA purification kit (MACHEREY-NAGEL) according to the manufacturer's instructions. Whole exome sequencing was performed by BGI with 100x sequencing depth. The data was analysed using IGW (Integrative Genomics Viewer) software. Genomic positions with at least 8-fold coverage across all cell lines were selected for consideration in the search for candidate variables. We searched for de novo SNP and

indel variables for both the mother and the child. Finally, we narrowed down the massive amount of sequencing data to a short list of variable positions in disease-relevant genes. The presence of SNPs and indels at the selected positions must be validated in the genomic DNA from the peripheral blood of each individual to show that the found variables are not the result of reprogramming. We did not find mutations on the intact DiGeorge allele except in TBX1 gene, where some variations were detectable in all the family members. Moreover there are *de novo* mutations in the DiGeorge patients at the other parts of the genome (1. table).

Gene	Type	Func	Exonic.Biotype	Impact	Chr	Ref	Observed	Cell line	Sanger seq validation from PBMC
TBX1a	SNP	missense_variant	MISSENSE	MODERATE	chr22	C	T	**GM, F, M**	GM, F:C/T ; M: only T
TBX1b	SNP	structural_interaction_variant	protein_coding	HIGH	chr22	T	C	**F, GF, P**	F: T/C ; GF, P: only C
NOTCH2	de novo indel	frameshift_variant	protein_coding	HIGH	chr1	CGG	C	**M**	
ADAMTS7	de novo indel	splice_donor_variant+intron_variant	protein_coding	HIGH	chr15	AC	A	**M**	
RAP1B	de novo SNP	structural_interaction_variant	protein_coding	HIGH	chr12	A	G	**M**	
CACNB2	de novo indel	splice_acceptor_variant+intron_variant	protein_coding	HIGH	chr10	T	TTTTTG	**P**	no conclusive result (sequencing problem - repetitive sequences)
NOTCH3	de novo SNP	missense_variant	MISSENSE	MODERATE	chr19	A	C	**P**	
DTX2	SNP/de novo SNP	missense_variant	MISSENSE	MODERATE	chr7	A	G	**GM, P**	
TYMP	SNP/de novo SNP	structural_interaction_variant	protein_coding	HIGH	chr22	G	A	**GF, M*, P**	GF, M, P: G/A + F: G homozygous
FGF1	SNP	structural_interaction_variant	protein_coding	HIGH	chr5	C	T	**GF, M, P**	GF, M, P: C/T
ASB11	SNP	structural_interaction_variant	protein_coding	HIGH	chrX	T	A	**GF, M, P**	GF: A (hemizig.); M, P: T/A + F: T (hemizig.)
PDCD1	indel	frameshift_variant	protein_coding	HIGH	chr19	G	GC	**P**	P: GC
NGFR	SNP	missense_variant	MISSENSE	MODERATE	chr17	C	T	**GF, M, P**	GF, M, P: C/T

Table 1. Whole Exome Sequencing (WES) data: de novo mutations in the iPSC lines of the family From the selected genetic variations (first column) we have validated the genes highlighted by green background. (The blue background shows the genes with pseudo genes and red is for the problematic primer design.). The last column shows the validation of the found variation from the peripheral blood samples.

We have also found all of the selected mutations in iPSCs and PBMCs. Next, we will optimise the protocol for the remaining genes (including CACNB2) and perform validation experiments.

After validating the exome sequencing and mRNA sequencing data, we perform a comparative analysis to determine the possible signalling pathways underlying the in vitro phenotype of the patients' (mother's and child's) endothelial cells. It may help to understand the genetic events that lead to intergenerational exacerbations of the disease.

We have a lot of experience with cardiomyocyte-endothelial cell co-cultures. In addition, endothelial cell-pericyte and endothelial-smooth muscle cell co-cultures were also prepared from healthy cell lines as preliminary efforts for co-culture experiments for the DiGeorge project. So far, the material has already been presented in the form of two PhD theses; further peer-reviewed original publications will follow these. Furthermore, in the framework of an international collaboration (with Thomas Eschenhagen's lab- University Medical Center Hamburg), we established 3-dimensional myocardial and myocardial-endothelial cultures from DiGeorge patients (unfortunately, there were significant delays in this work due to the pandemic).

6, Studies on DGCR8

DGCR8 is one of the missing genes in 22q11.2 microdeletion (most common microdeletion syndrome) and cannot cause all the disease-specific phenotypes; however, its role in the maturation of miRNAs and cell differentiation is well established in animal models. As there is no documented human iPSC line lacking the DGCR8 gene, our goal was to establish DGCR8 hemizygous and homozygous iPSC lines from healthy control cells using CRISPR technology. Based on animal experiments, we modified exon 3 in the DGCR8 gene by introducing a CAG-GFP-CAG puromycin cassette by employing Cas9 endonuclease targeted to the central region of exon 3 and utilising the non-homologous end joining mechanism. After transfection, we selected the transgene expressing iPSCs by antibiotic selection and then cell sorting for GFP signal, and following single-cell cloning, we generated iPSC clones. We have successfully established a clone with one copy of DGCR8; however, we could not find any homozygous KO clone. To validate DGCR8 elimination, the copy number and the DNA sequence of GFP integration were determined, and DGCR8 mRNA and protein levels were measured. We found that the mRNA level of DGCR8 had decreased by 20%, while the protein levels varied between 40-60% of the control, showing the successful deletion of DGCR8. We have also checked the pluripotent state of the clone (pluripotency markers by FACS and ICC, spontaneous differentiation capacity and normal karyotype) and published our result in Stem Cell Research (Ree, Borsy et al. 2020).

Maturation of microRNAs (miRNAs) begins by the "Microprocessor" complex, containing the Drosha endonuclease and its partner protein, "DiGeorge Syndrome Critical Region 8" (DGCR8). Although the main function of the two proteins is to coordinate the first step of precursor miRNAs formation, several studies revealed their miRNA-independent functions in other RNA-related pathways (e.g., in snoRNA decay) or, for the DGCR8, the role in tissue development. To investigate the specific roles of DGCR8 in various cellular pathways, we previously established a human embryonic stem-cell (hESC) line carrying a monoallelic DGCR8 mutation by using the CRISPR-Cas9 system. In this study, we generated four single-cell originated progenies without GFP and puromycin transgenes. We genetically characterised of the cell line and showed that DGCR8 heterozygous mutation results in only a modest effect on the mRNA level but a significant decrease at the protein level. Self-renewal and trilineage differentiation capacity of these hESCs were not affected by the mutation. However, partial disturbance of the Microprocessor function could be revealed in pri-miRNA processing along the human chromosome 19 miRNA cluster in several clones. With all these studies, we can demonstrate that the mutant hESC line is a good model to study not only miRNA-related but also other "noncanonical" functions of the DGCR8 protein. We published our results in Genes (Ree, Fothi et al. 2022).

The next steps could be a detailed investigation of the directed differentiation of DGCR8 KO lines, especially the cardiac and endothelial direction, as well as microRNA regulation.

7, Publications:

During this project, we also made some effort to validate pluripotent stem cell-based methods in basic research and disease modelling.

We developed an image-based optical-flow analysis, which applies a speckle tracking algorithm to videomicroscopy of hPSC-derived cardiomyocytes (hPSC-CM). This non-invasive method quantitatively assesses the dynamics of mechanical contraction of the CMs (such as contraction velocity field, beat rate, contractile strain and contraction-relaxation strain rate profile), which are important phenotypic characteristics of CMs and help to follow the in vitro differentiation process and find differences between patient-derived and healthy CMS (Izadifar, Berecz et al. 2022). Using the techniques we have developed during this project, we aimed to compare cardiac cell lines to primary cardiomyocytes and to mature cardiac tissues systematically (Onodi, Visnovitz et al. 2022), we determined new reference genes for gene expression studies (Racz, Nagy et al. 2021) and revealed the role of YAP1-TAZ of Hippo signalling in doxorubicin-induced cardiomyopathy (Berecz, Yiu et al. 2022). The objectives included the study of cell-cell interactions. At the Heart and Vascular Center, Semmelweis University, we made available a new automated high content confocal microscope system (Opera, PerkinElmer) funded by other sources. Cell viability was quantified in line with our original aims by the combined labelling of Topro3 necrosis marker, caspase 3/7, TMRM and Hoechst (nuclear stain, pyknosis) (Berecz, Yiu et al. 2022). New image processing algorithms for the fluorescence analysis of living and fixed cells have been developed, and the results were partially published (Majid, Orsolits et al. 2022). Our experiments used endothelial cells differentiated from certain hiPSC lines and compared their phenotypes. The group has provided in vivo, ex vivo and in vitro evidence that hESC and hiPSC-derived endothelial cells develop high-fidelity functional phenotype and thus can be used in angiogenesis assays. Human PSC-EC develop antifibrotic, vasoactive and anti-inflammatory properties on predefined matrices, supporting patient-specific vascular disease modelling, like those in DiGeorge syndrome (Gara, Zucchelli et al. 2022).

Endothelial-myocardial and endothelial-pericyte co-cultures were established during this project period in 2D and 3D systems (1. spheroid; 2. synthetic extracellular matrix; 3. hanging droplet and Aggrewell systems, respectively). In this paper we describe many currently used or developed approaches to stem cell-based disease modelling, particularly focusing on 3D cell culture models (Orsolits, Kovacs et al. 2021).

We also used our knowledge for further validation of iPSC-based modelling in the case of cardiovascular diseases (Marquez, Rodriguez et al. 1987, Szabo, Ree et al. 2020, Ontoria-Oviedo, Foldes et al. 2021, Orsolits, Kovacs et al. 2021, Bors, Orsolits et al. 2022). A method article describes our developed cell culture platforms for vascular phenotyping. It also details the methods leveraged for the DiGeorge program (Majid, Orsolits et al. 2022). We also established models for inflammation (Lilienberg, Apati et al. 2022), as the immune system can also be involved in DiGeorge syndrome. We have summarised our knowledge about 3D cardiovascular models and provided methodological strategies to accelerate their development (Zucchelli, Majid et al. 2019, Mitrecic, Hribljan et al. 2022).

Publication list:

1. Apáti Á, Varga N, Berecz T, Erdei Zs, Homolya L, Sarkadi B. Application of human pluripotent stem cells and pluripotent stem cell-derived cellular models for assessing drug toxicity. EXPERT OPINION ON DRUG METABOLISM & TOXICOLOGY (2019); 15: (1) pp. 61-75. IF: 3.532
2. Reé D, Borsy A, Fóthi Á, Orbán TI, Várady G, Erdei Z, Sarkadi B, Réthelyi J, Varga N, Apáti Á. Establishing a human embryonic stem cell clone with a heterozygous mutation in the DGCR8 gene. Stem Cell Res. 2020 Dec 22;50:102134, PMID: 33360445 IF: 2.02
3. Izadifar M, Berecz T, Apáti Á, Nagy A. An Optical-Flow-Based Method to Quantify Dynamic Behavior of Human Pluripotent Stem Cell-Derived Cardiomyocytes in Disease Modeling Platforms. Methods Mol Biol. 2022;2454:213-230. doi: 10.1007/7651_2021_382. PMID: 33982275. IF: 1.37 (2021)
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Personal notes on the conditions of this research project

The NKFIH-OTKA grant provided important support for the work described in this project, while the conditions for using this support were less than optimal. In the second year of the project, the COVID-19 pandemic broke out, and for more than six months, all experimental activities were stopped or strongly reduced. Since most of the conferences were cancelled or took place online during this period, the planned expenses for participating in the conferences were classified as material costs. In addition, the purchase orders and deliveries also were limited and unpredictable in the long run. This is one of the explanations for the high number of review articles of our works. On the positive side, this project supported obtaining the successful diplomas of three MSc students and provided the opportunity to submit a PhD thesis for one student, and two more are in progress.

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