XIV Annual Meeting of the Mediterranean Society for Reproductive Medicine (MSRM)
April 21-24 2016
Cesme Sheraton Hotel
Izmir, Turkey

The future of PGD and PGS: New technologies and gazing into the crystal ball

Muhterem Bahçe MD PhD

Gazing into the cyristal ball?

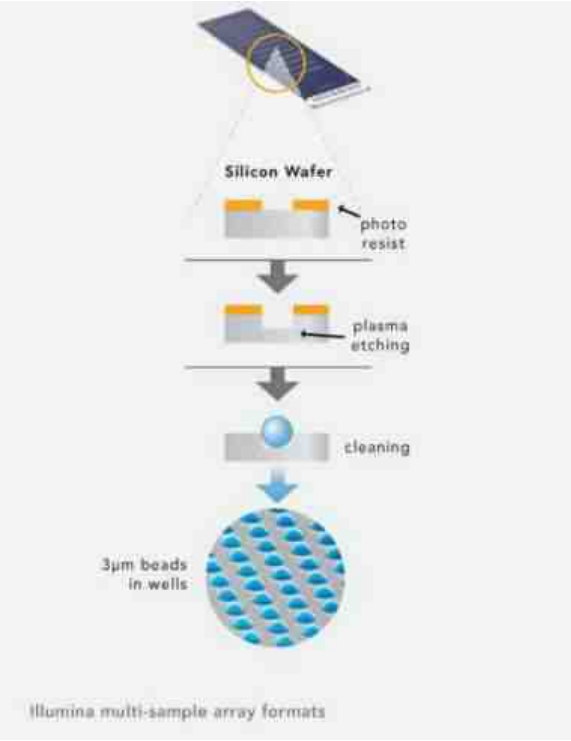
FUTURE?

- ACCURACY
- NON INVASIVE TECHNIQUES
- THERAPY/GENE EDITING

ACCURACY

- NEW TECHNOLOGIES
  - MORE INFORMATION
  - MORE DETAIL
  - MORE PRECISE

Illumina (Solexa) sequencing
Roche 454 sequencing
Ion torrent: Proton / PGM sequencing
SOLiD sequencing



Formats & Specifications

Arrays per Slide x Features per Array	1 x 2.1 million	3 x 720,000	1 x 385,000	4 x 72,000

"We are entering a golden age of genetics applied to the understanding of infertility, early human development and the diagnosis of inherited disease. Several other major technical advances are now on the verge of routine clinical application and the landscape of IVF is likely to be radically altered in the coming months and years."

Dagan Wells, 2014

ACCURACY

- UNDERSTANDING THE MOSAICISM AND THE EFFECTS ON EMBRYO
  - OUTCOMES OF TRANSFERRED EMBRYOS
    - SHORT TERM
    - MID TERM
    - LONG TERM

Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts

Ermanno Greco, M.D., Maria Giulia Minasi, M.Sc.; European Hospital, Rome, Italy
Francesco Fiorentino, Ph.D.; Genoma Molecular Genetics Laboratory Rome, Italy

Table 1. Clinical Outcomes of Single Mosaic Blastocysts Transferred.*

Patient No.	Chromosomal Constitution	Mosaicism†	Karyotype‡	Clinical Outcome
		percent		
1	arr(4)x1,(10)x1	40	46,XX	Baby healthy at birth
2	arr(6)x1,(15)x1	50	46,XX	Baby healthy at birth
3	arr(2)x1	40	46,XX	Baby healthy at birth
4	arr(2)x1	35	46,XY	Baby healthy at birth
5	arr(5)x1	50	46,XX	Baby healthy at birth
6	arr(5)x1,(7)x1	40	46,XX	Baby healthy at birth
7	arr(11)x1,(20)x3,(21)x3	30	NA	No pregnancy
8	arr(1)x1,(6)x3,(10)x3,(12)x3,(13)x3,(14)x3,(21)x3	50	NA	No pregnancy
9	arr(3)x1,(10)x3,(21)x3	35	NA	No pregnancy
10	arr(1)x3	50	NA	Biochemical pregnancy§
11	arr 9p21.2q34.3(26,609,645-140,499,771)x3	45	NA	Biochemical pregnancy§
12	arr(15)x3	30	NA	No pregnancy
13	arr(18)x1	50	NA	No pregnancy
14	arr(18)x1	50	NA	No pregnancy
15	arr(18)x1	40	NA	No pregnancy
16	arr(4)x1	50	NA	No pregnancy
17	arr(5)x3	40	NA	No pregnancy
18	arr 10q21.3q26.3(67,216,644-134,326,648)x3	50	NA	No pregnancy

* NA denotes not available.
† The approximate percentage of aneuploid cells in the transferred blastocyst is listed (see the Supplementary Appendix).
‡ The karyotype was determined by means of chorionic-villus sampling.
§ Biochemical pregnancy was defined by the presence of a low peak in levels of the beta subunit of human chorionic gonadotropin (β-hCG) (<100 mIU per milliliter), a rapid decrease in the urinary or serum β-hCG concentration, and no substantial delay in the onset of the next menstrual period, but with no detection of an identifiable pregnancy by means of ultrasonographic examination.

Mol Cytogenet. 2014 Sep 25;7(1):65. doi: 10.1186/s13039-014-0065-8. eCollection 2014.

Partial and complete trisomy 14 mosaicism: clinical follow-up, cytogenetic and molecular analysis.

Salas-Labadía C[1], Lieberman E[2], Cruz-Alcívar R[1], Navarrete-Meneses P[1], Gómez S[2], Cantú-Reyna C[3], Buiting K[4], Durán-McKinster C[5], Pérez-Vera P[1].

Prenat Diagn. 2001 Jun;21(6):457-60

Prenatal diagnosis of mosaicism for triploidy and trisomy 13.

Phelan MC[1], Curtis Rogers R, Michaelis RC, Moore CL, Blackburn W.

Rev Neurol. 2014 Aug 16;59(4):158-63.

Diploid/triploid mosaicism: a variable but characteristic phenotype.

Natera-De Benito D[1], Poo P, Gean E, Vicente-Villa A, García-Cazorla A, Fons-Estupiña MC.

Genet Couns. 2014;25(2):177-82.

Mosaic double aneuploidy (45,X/47,XX,+8) with aortic dissection.

Lee MN, Choi KH, Kim DK, Kim SH.

TABLE 1

Comparative genomic hybridization microarray results from 3 different cells from the same embryo.

Embryo #	Single blastomere	Blastocoel fluid DNA	Inner cell mass and trophectoderm
Euploid embryos (n = 23)			
1	46, XX	46, XX	46, XX
2	46, XX	46, XX	46, XX
3	46, XX	46, XX	46, XX
4	46, XX	46, XX	46, XX
5	46, XY	46, XY	46, XY
6	46, XX	46, XX	46, XX
7	No blastomere cell	46, XX	46, XX
8		46, XX	46, XX
9		46, XY	46, XY
10		46, XY	46, XY
11		46, XY	46, XY
12		46, XY	46, XY
13		46, XY	46, XY
14		46, XY	46, XY
15		46, XY	46, XY
16		46, XY	46, XY
17		46, XY	46, XY
18		46, XX	46, XX
19		46, XX	46, XX
20		46, XY	46, XY
21		46, XY	46, XY
22		46, XY	46, XY
23		46, XX	46, XX
Aneuploid embryos (n = 4)			
1	No blastomere cell	45, XY, −21	45, XY, −21
2		45, XX, −13	45, XX, −13
3		46, XY, −11, +20	46, XY, −11, +20
4		47, XX, +19	47, XX, +19
Aneusomy (n = 21)			
1	46, XY	45, XY, −6	46, XY
2	46, XY	45, X	46, XY
3	46, XY	45, X	46,XY
4	45, XX, −15	46, XX	46, XX
5	45, XY, +18	46, XY	46, XY
6	45, XY, −12	47, XY, +12	45, XY, −12
7	46, XX, +16, −20	43, XX, −3, −7, −16, +20, −22	46, XX, +16, −20
8	47, XY, +13	46, XY	47, XY, +13
9	45, XY, −11, +20, −21	45, XY, −11, +20	46, XY, −11, +20, −21
10	48, XY, +6, +15	48, XY, +15, +18	48, XY, +6, +15
11	49, XX, +1, +5, +15	48, XX, +5, +15	47, XX, +22
12	No blastomere cell	45, XX, −16	46, XX
13		53, XYY, +2, +4, +6, +7, +9, +20	46, XY
14		45, XY, −20	46, XY
15		49, XX, +13, +15, +19	44, XX, −16, −22
16		47, XX, +15	45, XX, −21
17		46, XX, −13, +16	46, XX, +5, −16
18		46, XX	46, XX, −13, +17
19		53, XYY	46, XY
20		48, XY, −1, +10, +12, +22	47, XY, +17
21		48, XY, +15, +18	48, XY, +6, +15
Resolution of aneusomy during differentiation from an aneuploid cleavage-stage embryo to a euploid blastocyst with aneuploid DNA within the blastocoel fluid (n = 12)			
1	45, X	47, XXX	46, XX
2	47, XX, +1	47, XX, +1	46, XX
3	47, XX, +13	45, XX, −13	46, XX
4	47, XX, +16	47, XX, +16	46, XX
5	47, XX, +17	45, X	46, XX
6	47, XX, +7	48, XX, +7, +17	46, XX
7	46, XY, +1, −10	48, XY, −1, +10, +12, +22	46, XY
8	47, XY, +5	48, XY, +5, +21	46, XY
9	48, XY, +17, +18	47, XY, +17	46, XY
10	48, XY, +8, +9	47, XY, +9	46, XY
11	46, XX, +19, −21	46, XX, +19, −21	46, XX
12	50, XX, +1, +2, +5, +22	49, XX, +2, +5, +19	46, XX

Tobler. Blastocoel fluid harbors embryonic DNA. Fertil Steril 2015.

Blastocoel fluid from differentiated blastocysts harbors embryonic genomic material capable of a whole-genome deoxyribonucleic acid amplification and comprehensive chromosome microarray analysis

Kyle J. Tobler, M.D.,[a,b] Yulian Zhao, Ph.D., M.D., M.B.A.,[a] Ric Ross, M.S.,[c] Andy T. Benner, M.S.,[d] Xin Xu, M.S.,[d] Luke Du, M.D.,[d] Kathleen Broman, B.S.,[a] Kim Thrift, B.S.,[a] Paul R. Brezina, M.D., M.B.A.,[e,f,g] and William G. Kearns, Ph.D.[e,g]

[a] Division of Reproductive Endocrinology and Infertility, Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, Maryland; [b] Department of Obstetrics and Gynecology, Womack Army Medical Center, Fort Bragg, North Carolina; [c] Fort Worth Fertility, Fort Worth, Texas; [d] AdvaGenix, LLC, Rockville, Maryland; [e] Division of Reproductive Endocrinology and Infertility, Department of Gynecology and Obstetrics, Vanderbilt University School of Medicine; [f] Department of Surgery, St. Jude Children's Research Hospital; and [g] Fertility Associates of Memphis, Memphis, Tennessee

TABLE 2

Both the molecular karyotype and ploidy (aneuploid vs. euploid) status of BF-DNA, compared with the ICM-TE of all embryos and the quantitative parameters for the diagnostic accuracy of BF-DNA to represent the ICM-TE (whole embryo).

Variable	Data
Concordant karyotypes	48% (29/60)
Discordant karyotypes	52% (31/60)
Sensitivity	0.88 (95% CI: 0.62–0.98)
Specificity	0.55 (95% CI: 0.39–0.70)
Positive predictive value	0.41 (95% CI: 0.25–0.60)
Negative predictive value	0.92 (95% CI: 0.75–0.99)

Tobler. Blastocoel fluid harbors embryonic DNA. Fertil Steril 2015.

In conclusion, the results reported in the present study reinforce the potential use of BF as a source of DNA for PGS or PGD for translocations, although the reason for the detected discordances has not been yet demonstrated to be related to the biology of preimplantation embryos. It is worth noting that BF aspiration, being less invasive than alternative methods for the sampling of embryo DNA, has potential cost advantages. Highly experienced staff, trained in embryo biopsy, are probably not essential, and costly laser equipment is not needed. Needless to say, the proportion of samples producing DNA suitable for a-CGH analysis needs to be optimized.

Preimplantation genetic testing: polar bodies, blastomeres, trophectoderm cells, or blastocoelic fluid?

M. Cristina Magli, M.Sc., Alessandra Pomante, Ph.D., Giulia Cafueri, B.Sc., Marzia Valerio, B.Sc., Andor Crippa, Ph.D., Anna P. Ferraretti, M.D., and Luca Gianaroli, M.D.

SISMER, Reproductive Medicine Unit, Bologna, Italy

TABLE 1

Cases with ploidy discordance between BF and the analysis done at previous stages and between BF and TE cells.

ID	PB1	PB2	Blastomere	Prediction of the blastocyst chromosome status	BF	TE cells	WE
33	Loss 6 Loss 22	Gain 22		Gain 6	Euploid	Euploid	Euploid
50	Loss 18 Loss 19	Gain 18 Loss 15		Gain 19 Gain 15	Euploid	Euploid	Euploid
45			Gain 9	Gain 9	Euploid	Loss 9	Loss 9
54			Loss 2	Loss 2	Euploid	No Result	Loss 2
60			Gain 10p11.23->10q26.3 Gain 20 Loss 16	Gain 10p11.23->10q26.3 Gain 20 Loss 16	Gain 10 Gain 20 Loss 16	Euploid	Gain 10 Gain 20 Loss 16

Note: In the first four sets, BFs were discordant with the results predicted by PBs (PB1 and PB2), but they were concordant with corresponding TE cells and with the WE. BFs were discordant also in the following two cases compared with the prediction made by blastomere analysis. In one of these cases, the WE had the same profile as the blastomere, while in the second both TE and WE gave the same result that involved the same chromosome predicted to be aneuploid by the blastomere, but with an opposite type of aneuploidy. In the last case, no. 60, the ploidy discordance involved TE cells that were euploid conversely to the chromosome status detected in all other biopsies. The term "Gain" indicates hyperhaploidy; the term "Loss" indicates hypohaploidy.

Magli. Blastocentesis: a source of DNA for PGT. Fertil Steril 2016.

Reprod Biomed Online. 2016 Feb 24.

Corona cell RNA sequencing from individual oocytes revealed transcripts and pathways linked to euploid oocyte competence and live birth.

Parks JC[1], Patton AL[2], McCallie BR[3], Griffin DK[4], Schoolcraft WB[5], Katz-Jaffe MG[6].

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Abstract

Corona cells surround the oocyte and maintain a close relationship through transzonal processes and gap junctions, and may be used to assess oocyte competence. In this study, the corona cell transcriptome of individual cumulus oocyte complexes (COCs) was investigated. Isolated corona cells were collected from COCs that developed into euploid blastocysts and were transferred in a subsequent frozen embryo transfer. Ten corona cell samples underwent RNA-sequencing to generate unique gene expression profiles. Live birth was compared with negative implantation after the transfer of a euploid blastocyst using bioinformatics and statistical analysis. Individual corona cell samples produced a mean of 21.2 million sequence reads, and **307 differentially expressed transcrpits** ($P < 0.05$; fold change ≥2). Enriched pathway analysis showed Wnt signalling, mitogen-activated protein kinases signalling, focal adhesion and tricarboxylic acid cycle to be affected by implantation outcome. The Wnt/beta-catenin signalling pathway, including genes **APC, AXIN and GSK3B**, were independently validated by real-time quantitative reverse transcription. Individual, corona cell transcriptome was successfully generated using RNA-sequencing. Key genes and signalling pathways were identified in association with implantation outcome after the transfer of a euploid blastocyst in a frozen embryo transfer. **These data could provide novel biomarkers for the non-invasive assessment of embryo viability.**

Hindawi Publishing Corporation
Stem Cells International
Volume 2016, Article ID 3984937, 17 pages
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Review Article

MicroRNAs: From Female Fertility, Germ Cells, and Stem Cells to Cancer in Humans

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[2]*Sahlgrenska Cancer Center, Department of Pathology, Institute of Biomedicine, University of Gothenburg, 40530 Gothenburg, Sweden*
[3]*TATAA Biocenter, 41103 Gothenburg, Sweden*
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[5]*Institute for Anatomy and Cell Biology, University of Heidelberg, 69120 Heidelberg, Germany*

- Discovery of exosomes, cell-derived vesicles, which are released from the cell when multivesicular bodies fuse with the plasma membrane or are released directly from the plasma membrane . Exosomes can carry different proteins but also miRNAs and mRNAs between different cells and are deeply involved in cell-to-cell communication. They can be found in almost all body fluids and also in media of cell cultures. Exosomes have a great potential to be used for prognosis, therapy, and biomarkers of different diseases including infertility.
- It is known that miRNAs are a family of naturally occurring small noncoding RNA molecules of 19–24 nucleotides in length that play an important regulatory role in gene expression

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MicroRNAs: From Female Fertility, Germ Cells, and Stem Cells to Cancer in Humans

Irma Virant-Klun,[1] Anders Ståhlberg,[2] Mikael Kubista,[3,4] and Thomas Skutella[5]

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•There were some miRNAs, differently expressed between euploid and aneuploid embryos, such as miR-141, miR-27b, miR-339-3p, and miR-345 that were all upregulated in euploid embryos.

•miR-191 was more highly concentrated in media from aneuploid embryos, and miR- 191, miR-372, and miR-645 were more highly concentrated in media from failed in vitro fertilization cycles without pregnancy.

•It was found that ten miRNAs were overexpressed in RIF endometrial samples, including miR-23b*, miR-99a, and* miR-145, whereas three were underexpressed.

REPRODUCTIVE BIOLOGY

Aberration of blastocyst microRNA expression is associated with human infertility

Blair McCallie, B.Sc.,[a] William B. Schoolcraft, M.D.,[b] and Mandy G. Katz-Jaffe, Ph.D.[a,b]

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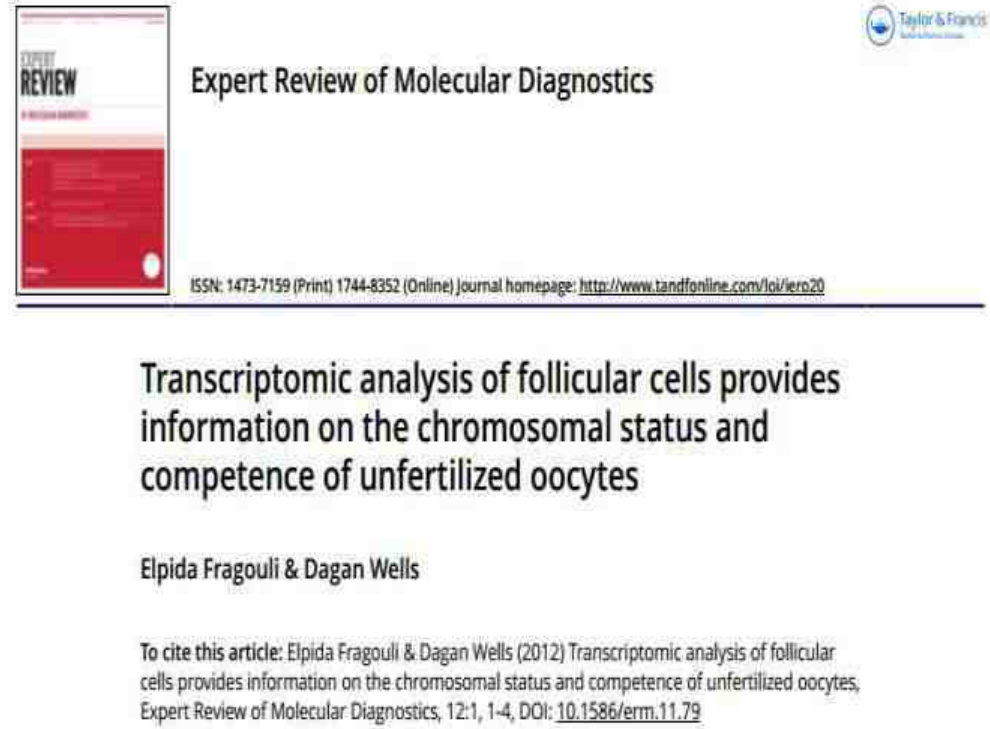
- When analyzed in regard to infertility diagnosis, a potential association was observed with blastocysts derived from patients with male factor infertility or polycystic ovaries displaying specific aberrant miRNA profiles when compared with blastocysts derived from fertile donor oocyte control cycles.
- An assumption that morphologically similar, transferable quality blastocysts will have the same implantation potential is not always reflective of clinical outcome. Aberrant expression of blastocyst miRNAs could possibly be considered as an additional contributing factor to implantation failure in correlation with specific infertility diagnosis.

- However, it remains the case that most transcriptomic studies undertaken to find novel CC biomarkers have identified different candidate genes. The lack of uniformity or overlap between studies suggests that there may be confounding variables affecting gene expression, perhaps of a patient, clinic, treatment or etiology-specific nature.

- “A noninvasive test based on cumulus cells, allowing the identification of the embryo with the highest potential to survive and implant, would revolutionize assisted reproduction techniques...”



Expert Review of Molecular Diagnostics

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Transcriptomic analysis of follicular cells provides information on the chromosomal status and competence of unfertilized oocytes

Elpida Fragouli & Dagan Wells

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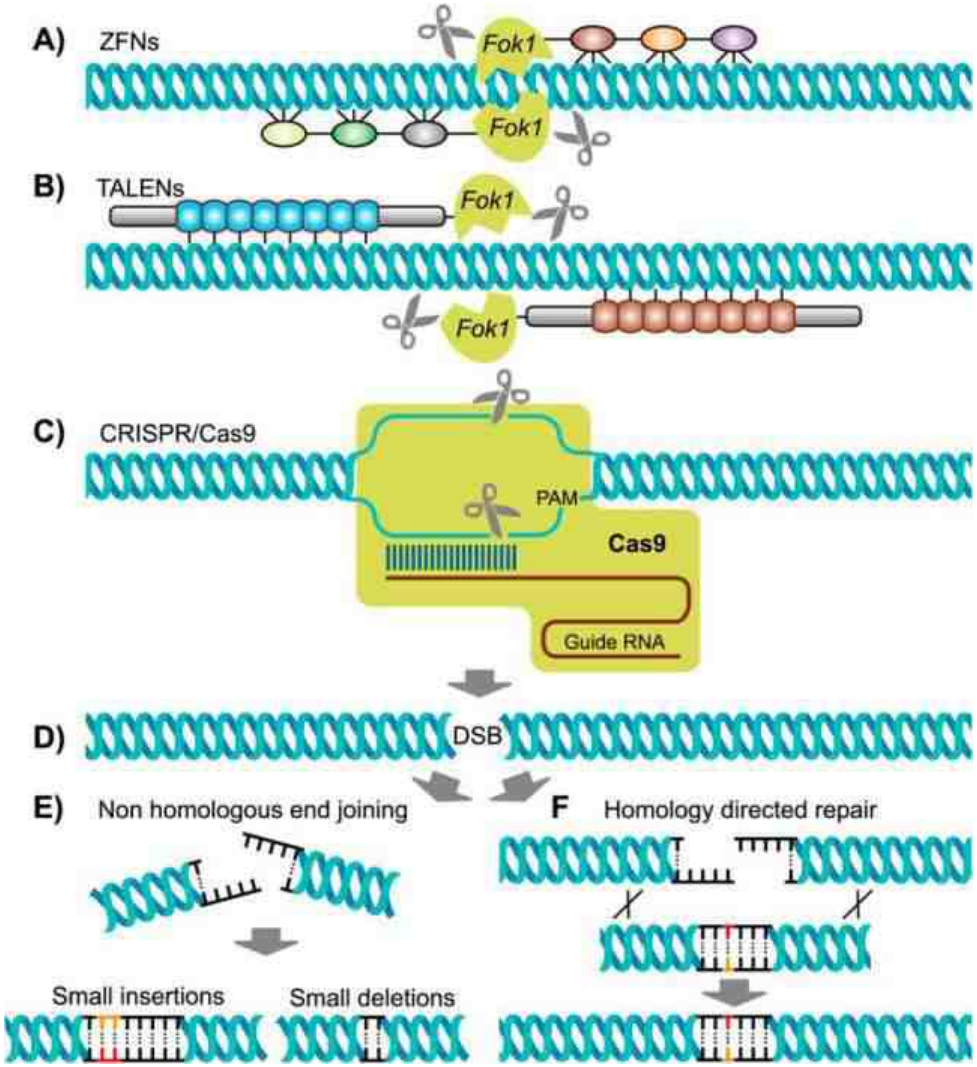
Research Article

EMBO Molecular Medicine

Modelling and rescuing neurodevelopmental defect of Down syndrome using induced pluripotent stem cells from monozygotic twins discordant for trisomy 21

Youssef Hibaoui[1,2], Iwona Grad[1], Audrey Letourneau[2], M Reza Sailani[2], Sophie Dahoun[2], Federico A Santoni[2], Stefania Gimelli[2], Michel Guipponi[2], Marie Françoise Pelte[3], Frédérique Béna[2], Stylianos E Antonarakis[2,4,*] & Anis Feki[1,5,**]

- The generation of iPSCs from monozygotic twins discordant for trisomy 21 is an innovative way to study DS neurodevelopment as it offers an unprecedented opportunity to study early embryonic development and enables the investigation of the detailed pathogenetic mechanisms by which the extra copy of HSA21 leads to DS phenotypes. The finding that DYRK1A inhibition by pharmacological and genetic approaches contributes to reversal of the abnormal neurogenesis in NPCs and neurons derived from DS iPSCs, allows a proof-of principle for potential screening tests using iPSC technology and should provide the basis for designing new therapeutic approaches for DS patients.



SCIENTIFIC REPORTS

OPEN

Efficient introgression of allelic variants by embryo-mediated editing of the bovine genome

Received: 04 December 2014
Accepted: 12 May 2015
Published: 09 July 2015

Jingwei Wei[1,2], Stefan Wagner[1], Dan Lu[1,3], Paul Maclean[1], Daniel F. Carlson[4], Scott C. Fahrenkrug[4] & Götz Laible[1]

Figure 1. Schematic outline of the application of the three most commonly used genome editors (ZFNs, TALENs, CRISPRs) to introduce targeted genome modifications into animal genomes. ZFNs (A) are chimeric designer proteins that combine a customizable DNA binding domain with the catalytic domain of the restriction endonuclease FokI (green). The DNA binding domain is comprised of several, modular ZFs (colored ellipsoids) that each can bind to a specific triplet of DNA sequence. ZFNs require the coordinated binding of a pair of ZFNs at the target site to gain activity of the dimerization-dependent FokI nuclease. TALENs (B) are following the same principle but are using entirely different DNA binding modules (TALE repeats, blue and brown elements) that each specifically bind a single nucleotide. CRISPR/Cas9 (C) uses a universal nuclease with two catalytic domains (Cas9, green) that is guided to its specific target site by a stretch of 20 nucleotides of sequence complementarity of the guide RNA to the target sequence (indicated as base-pairing between the single stranded target DNA and guide RNA) in addition to the juxtaposition to the protospacer adjacent motif (PAM). All three editors (A to C) have in common that the targeted nuclease will cleave both DNA strands (indicated by the scissors) resulting in a double strand break (DSB) at the target site (D). This DSB can be repaired by the endogenous cellular machinery by non homologous end joining (E) which is an error prone mechanism leading to the frequent introduction of small insertions (colored bases) or deletions. If a homologous template is provided the DSB can be resolved through homology directed repair (F) allowing for the knockin of template-specified sequence variations (novel, colored base pair).

Protein Cell 2015, 6(7):472–475
DOI 10.1007/s13238-015-0177-x

Protein & Cell

RESEARCH HIGHLIGHT

Eliminate mitochondrial diseases by gene editing in germ-line cells and embryos

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✉ Correspondence: fyi@stanford.edu (F. Yi), qujing@ioz.ac.cn (J. Qu)

- In recent years, remarkable advances in nuclease-based genome editing technologies including helper-dependent adenovirus vector (HDAdV), zinc finger nuclease (ZFN), transcriptional activator-like effector nucleases (TALEN), and the newly developed clustered regularly interspaced short palindromic repeats (CRISPR)/ Cas system, have offered unprecedented possibilities of precise gene editing in a variety of organisms, which is promising for not only basic researches but also therapeutic studies of human diseases.

744–760 Nucleic Acids Research, 2016, Vol. 44, No. 2 Published online 17 December 2015
doi: 10.1093/nar/gkv1464

piggyBac transposons expressing full-length human dystrophin enable genetic correction of dystrophic mesoangioblasts

Mariana Loperfido[1,2], Susan Jarmin[3], Sumitava Dastidar[1], Mario Di Matteo[1,2], Ilaria Perini[4], Marc Moore[3], Nisha Nair[1], Ermira Samara-Kuko[1], Takis Athanasopoulos[3,5], Francesco Saverio Tedesco[6], George Dickson[3,†], Maurilio Sampaolesi[4,†], Thierry VandenDriessche[1,2,†] and Marinee K. Chuah[1,2,*,†]

- In conclusion, this study provides the first evidence of PB-mediated human full-length dystrophin expression in dystrophic MABs for the treatment DMD. Further characterizations in vivo are needed upon transplantation of the PB transposon-modified MABs in the GRMD model, to ultimately justify a clinical study in patients suffering from DMD.

Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and *piggyBac*

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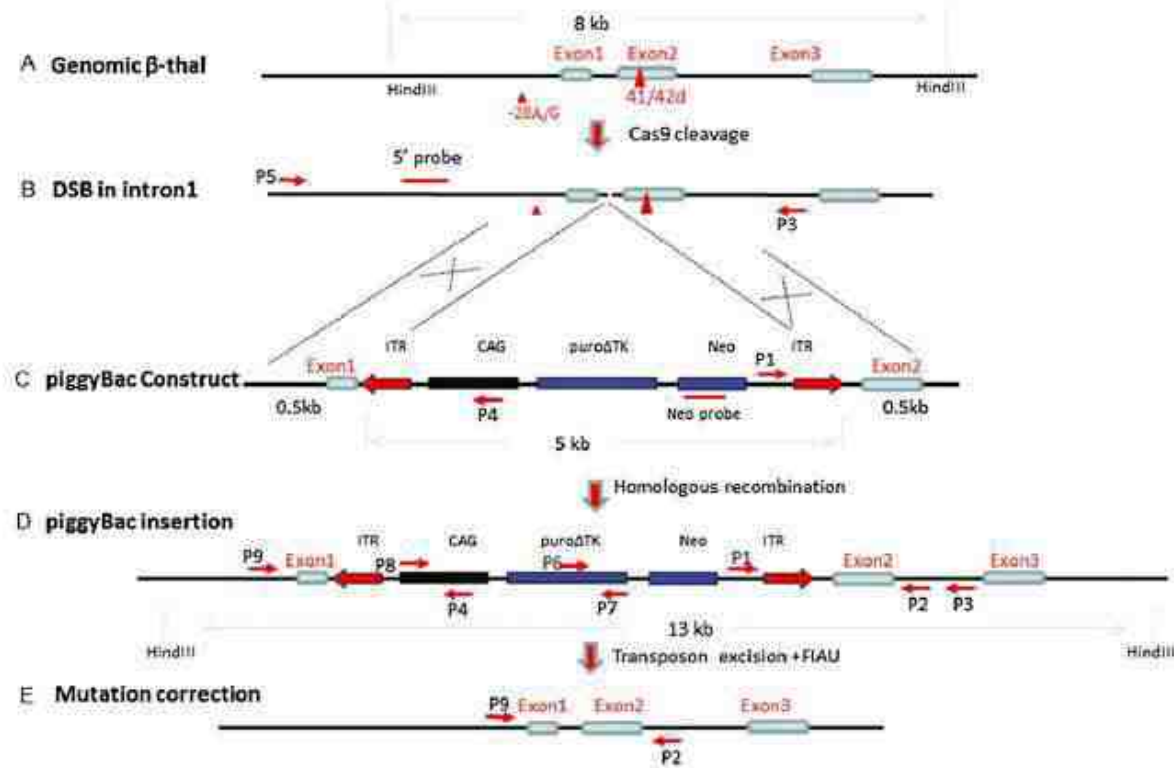

Figure 1. Strategy for seamless correction of the β-thalassemia mutations using *piggyBac* and Cas9. (*A*) Locations of the two mutations at −28 with A/G substitution and the codon 41/42 with 4-bp deletion. (*B*) The DSB at intron 1 following Cas9 cleavage. (*C*) The targeting construct of the *piggyBac* transposon carrying the selectable markers, *puroΔtk* and *Neo*, flanked by 500 bp of wild-type genomic sequences. (*D*) Insertion of the *piggyBac* following homologous recombination. (*E*) After selection with puromycin, clones with mutation-corrected lines were identified and transiently transfected with transposase expression plasmids, followed by treatment with FIAU to eliminate *piggyBac*-containing clones, and the seamless mutation-corrected clones were isolated.

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