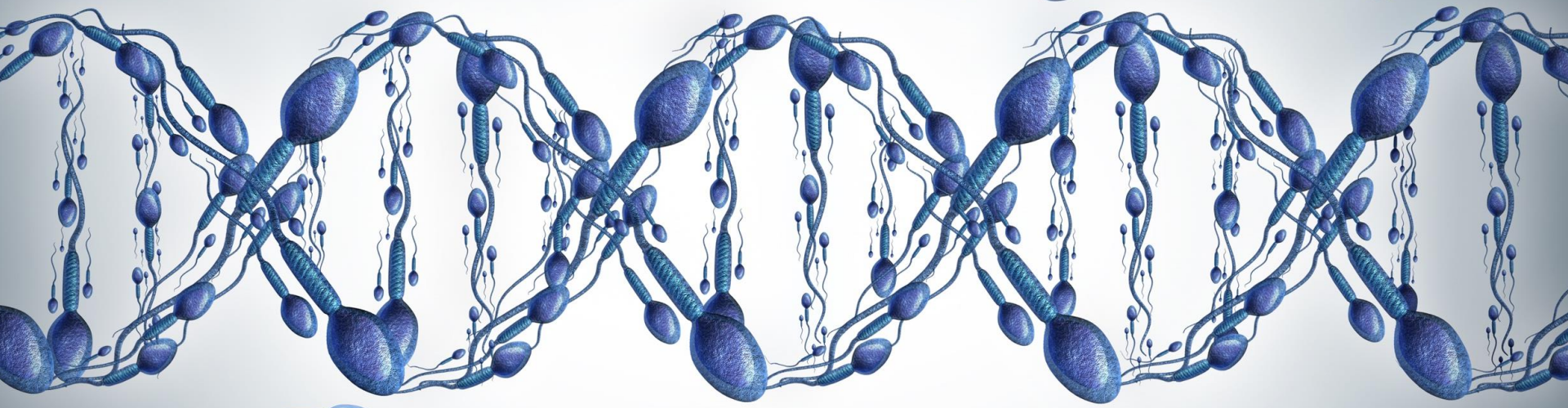
GENETICS AND EPIGENETICS OF MALE INFERTILITY



DOUG CARRELL, PHD, HCLD

DEPARTMENTS OF HUMAN GENETICS AND SURGERY (UROLOGY)

ANDROLOGY AND IVF LABORATORIES DIRECTOR

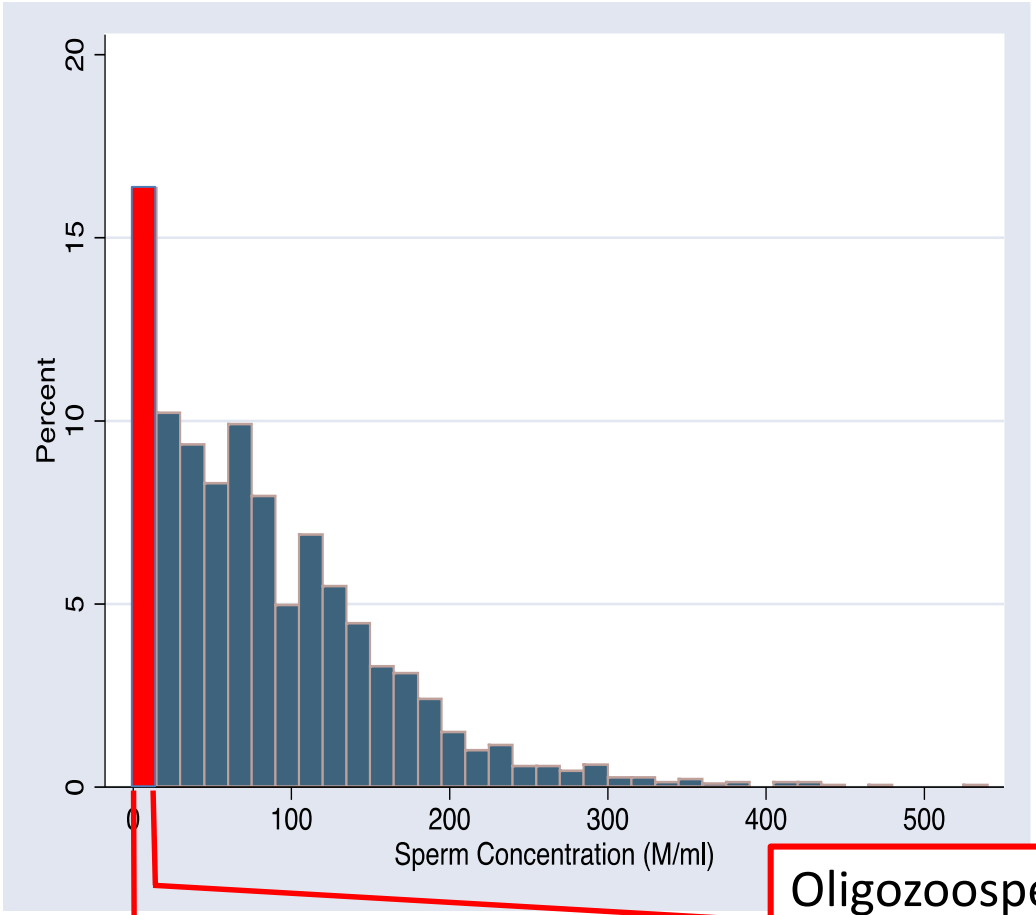
UNIVERSITY OF UTAH SCHOOL OF MEDICINE

OUTLINE

- Etiologies of azoospermia
- Overview of spermatogenesis complexity
- Genomic tools
- Current efforts

- Sperm epigenetics

MALE INFERTILITY IS COMMON



Semen analysis from 2280 Andrology patients at University of Utah

Normospermic

Spermatogenic Impairment

Oligozoospermia (<15M/ml; 15% prevalence)

Azoospermia (2% prevalence)

GENETIC CAUSES OF AZOOSPERMIA

The Journal of
CLINICAL
ENDOCRINOLOGY

VOLUME 2 NOVEMBER, 1942

Syndrome Characterized by Gynecomastia, Aspermatogenesis without A-Leydigism, and Increased Excretion of Follicle-Stimulating Hormone[1]

HARRY F. KLINEFELTER, JR.,[2] M.D., EDWARD C. REIFENSTEIN, JR., M.D. AND FULLER ALBRIGHT, M.D.

From the Medical Service of the Massachusetts General Hospital and the Department of Medicine of the Harvard Medical School, Boston, Massachusetts

302 NATURE January 31, 1959 VOL. 183

A CASE OF HUMAN INTERSEXUALITY HAVING A POSSIBLE XXY SEX-DETERMINING MECHANISM

By PATRICIA A. JACOBS and DR. J. A. STRONG

Medical Research Council Group for Research on the General Effects of Radiation and Department for Endocrine and Metabolic Diseases, Western General Hospital and University of Edinburgh

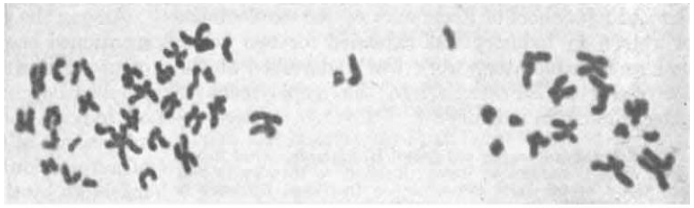


Fig. 1. Metaphase plate showing 47 chromosomes

~10-15% of NOA

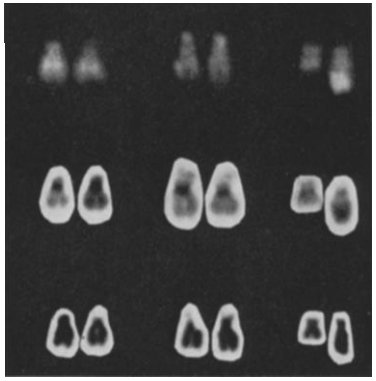
Hum. Genet. 34, 119—124 (1976)

Original Investigations

Localization of Factors Controlling Spermatogenesis in the Nonfluorescent Portion of the Human Y Chromosome Long Arm

L. Tiepolo and Orsetta Zuffardi

Institute of General Biology, Medical Faculty, University of Pavia, Italy



~10-15% of NOA

21 22 Y deleted/
Y normal

RARE GENETIC CAUSES OF NOA (CUMULATIVELY <5% OF CASES)

- Kallmann Syndrome- few mutations identified
- Robertsonian translocations
- XX males
- Point mutations/CNVs
  - USP26, SOX3, TEX11, TEX14, MEIOB, DNAH6, DAZL, DAX-1, DMRT1 etc.

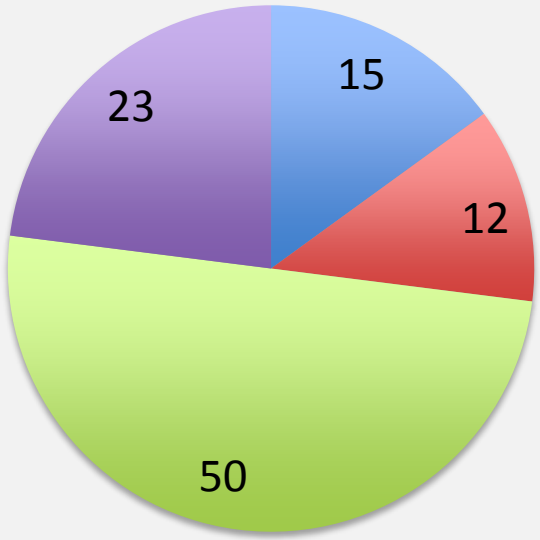
ETIOLOGIES OF MALE INFERTILITY

List of etiological factors involved in male factor infertility.

Congenital factors
-Anorchia
-Cryptorchidism
-Congenital Absence of Vas Deferens
-Genetic abnormalities (caryotype anomalies including Klinefelter syndrome; Y chromosome mirodeletions; Kallmann syndrome, mutations in genes involved in Hypothalamus–pituitary–gonadal axis, Partial/Mild Androgen Insensitivity syndrome)

Acquired factors
-Testis trauma
-Testicular torsion
-Post-inflammatory forms (orchitis, epididymitis)
-Obstruction, subobstruction of proximal and/or distal urogenital tract
-Recurrent urogenital infections, prostatitis, prostatovesciculitis
-Exogenous factors (medications, cytotoxic drugs, irradiation, heat etc)
-Systemic diseases (liver cirrhosis, renal failure etc)
-Varicocele (depending on the grade)
-Surgeries that can damage vascularisation of the testes
-Erectile, ejaculatory dysfunction
-Acquired hypogonadotrophic hypogonadism or endocrine factors

Idiopathic forms
-Unknown etiology (about 50%)



C. Krausz / Best Practice & Research Clinical Endocrinology & Metabolism 25 (2011) 271–285

EFFORTS TO CHARACTERIZE THE GENETICS OF MALE INFERTILITY

Gene Re-sequencing 2013

Endocrinopathies

GNRH KAL FSHB
AR FSH INSR
LH SRD5 CAG
LHR FSHR GGN
LIPE SBF1
ESR2 IL1RN

Metabolism/ Antioxidant

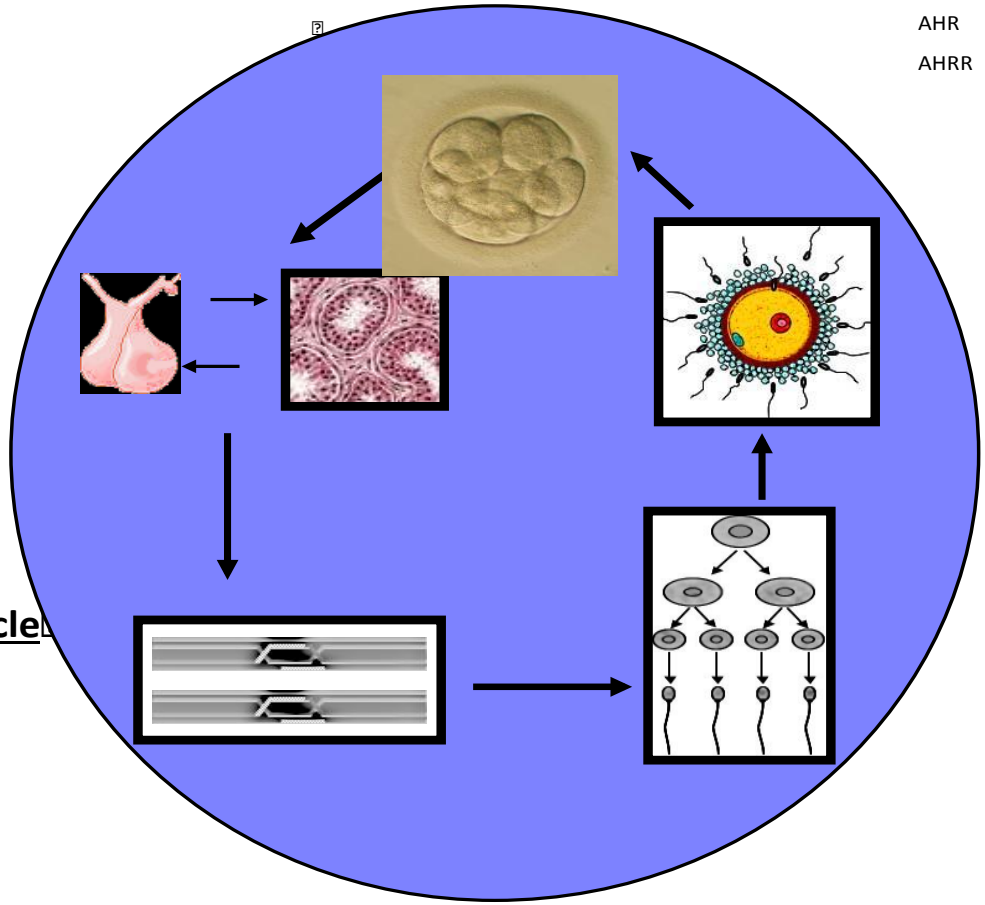
GSTM1 GSTT1 HMOX1
CYP1B1 EPHX2 CHDH
SOD2 NOS PON1

Meiotic/Cell Cycle

SPO11 DMC1 SCP3
MSH4 MLH1 SYCP3
BRCA2 ERCC1 FUS
FKBP6 LIMK2 MLH1
MEI1 MLH3 TP53
MSH5 REC8 MDM2
UBE2B HORMAD1 XRCC1

Development/Differentiation

SRY INSL3 DICER1 DROSHA SOX5
SOX9 LGR8 PEX10 SEPT12
CFTR KIT BMP4 BMP8



Sperm Function

PRM1 CSNK2A2 PICK1 DPY19L2
PRM2 APOB AKAP4 LOC203413
TNP1 ADAM2,3 CATSPER1-4
SPERM1 ROS PLA2G6 SEMG1
AHR ARNT PATZ1
AHRR ART3 EPPIN

Spermatogenesis

CREM ACT (FHL5) CLOCK
DAZL DDC H2BFWT
UBE2B DAZ3 DNMT3L
RBM BAX BRDT
ACE GOPC NANOS1
LIMK2 LMTK2 USP26
YBX2 NANOS3 TSPY
PARP2 PRDM9 SOHLH1
CSNK2A2 DDX25 MJD1A
H1FNT TAF7L TEX15
TBPL1 TNP2 GRTH
FASLG FAS CFTR
MTRR PYSSK4 PYGO2
IL1B MTHFR HSF2
TSSK2,4,6 UTP14 UBR2
TNF BCL2L2 TAS2R38
HMGB2 PPP1CC DR2W3
STYX PACRG KLK2
XPC PGAM4 ETV5 PMS2

SNP GENOME-WIDE ASSOCIATION STUDIES

Journal of Andrology, Vol. 30, No. 6, November/December 2009

Journal of ANDROLOGY

Genome-Wide Study of Single-Nucleotide Polymorphisms Associated With Azoospermia and Severe Oligozoospermia

KENNETH I. ASTON* AND DOUGLAS T. CARRELL*†‡

J Med Genet. 2012 January; 49(1): 58–65.
Published online 2011 December 3. doi: 10.1136/jmedgenet-2011-100174
Original article

Journal of MEDICAL GENETICS

A genome-wide association study of men with symptoms of testicular dysgenesis syndrome and its network biology interpretation

Marlene D Dalgaard,[1] Nils Weinhold,[2] Daniel Edsgärd,[2] Jeremy D Silver,[3] Tune H Pers,[2,4] John E Nielsen,[1] Niels Jørgensen,[1] Anders Juul,[1] Thomas A Gerds,[3] Aleksander Giwercman,[5] Yvonne L Giwercman,[5] Gabriella Cohn-Cedermark,[6] Helena E Virtanen,[7] Jorma Toppari,[7,8] Gedske Daugaard,[9] Thomas S Jensen,[2] Søren Brunak,[2] Ewa Rajpert-De Meyts,[1] Niels E Skakkebæk,[1] Henrik Leffers,[1,9] and Ramneek Gupta[2,10]

AJHG
Volume 90, Issue 5, 4 May 2012, Pages 900–906

Cell PRESS

Report

A Genome-wide Association Study Reveals that Variants within the HLA Region Are Associated with Risk for Nonobstructive Azoospermia

Han Zhao[1, 2, 3, 4, 19], Jianfeng Xu[5, 6, 7, 8, 19], Haobo Zhang[1, 2, 3, 19], Jielin Sun[7, 19], Yingpu Sun[9, 19], Zhong Wang[7], Jiayin Liu[10], Qiang Ding[6], Shaoming Lu[1, 4], Rong Shi[11], Li You[1, 2, 3, 4], Yingying Qin[1, 2, 3, 4], Xiaoming Zhao[12], Xiaoling Lin[5], Xiao Li[1, 4], Junjie Feng[7], Li Wang[1, 2, 3, 4], Jeffrey M. Trent[8], Chengyan Xu[1, 4], Ying Gao[13], Bo Zhang[14], Xuan Gao[1, 2, 3], Jingmei Hu[1, 2, 3], Hong Chen[1, 3, 4], Guangyu Li[1, 3, 4], Junzhao Zhao[15], Shuhua Zou[16], Hong Jiang[17], Cuifang Hao[18], Yueran Zhao[1, 2, 3, 4], Jinglong Ma[1, 2, 3, 4], S. Lilly Zheng[7], Zi-Jiang Chen[1, 2, 3, 4]

AJHG
Volume 90, Issue 6, 8 June 2012, Pages 950–961

Cell PRESS

Article

Genome-wide Association Study Identifies Candidate Genes for Male Fertility Traits in Humans

Gülüm Kosova[1, 4], Nicole M. Scott[1], Craig Niederberger[2], Gail S. Prins[2], Carole Ober[1, 3]

Human Reproduction, Vol.29, No.4 pp. 860–866, 2014
Advanced Access publication on February 18, 2014 doi:10.1093/humrep/deu013

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Comprehensive pathway-based analysis identifies associations of *BCL2*, *GNAO1* and *CHD2* with non-obstructive azoospermia risk

Yufeng Qin[1,2,†], Juan Ji[1,2,†], Guizhen Du[1,2], Wei Wu[1,2], Juncheng Dai[1], Zhibin Hu[1,2], Jiahao Sha[1], Bo Hang[3], Juncheng Lu[1,2,*], Yankai Xia[1,2], and Xinru Wang[1,2]

ELSEVIER
Mitochondrion
Volume 24, September 2015, Pages 87–92

MitoMatters

A genome-wide association study of mitochondrial DNA in Chinese men identifies two risk single nucleotide substitutions for idiopathic oligoasthenospermia

Juncheng Lu[a, b, 1], Miaofei Xu[a, b, 1], Rong Wang[c, 1], Yufeng Qin[a, b], Jing Ren[c], Wei Wu[a, b], Ling Song[a, b], Shoulin Wang[a, b], Zuomin Zhou[a], Hongbing Shen[a, d], Jiahao Sha[a], Zhibin Hu[a, d], Yankai Xia[a, b],

nature COMMUNICATIONS

ARTICLE
Received 23 Aug 2013 | Accepted 11 Apr 2014 | Published 23 May 2014 DOI: 10.1038/ncomms4857

Association analysis identifies new risk loci for non-obstructive azoospermia in Chinese men

Zhibin Hu[1,2,*], Zheng Li[3,*], Jun Yu[1,4,*], Chao Tong[5,*], Yuan Lin[1,2,*], Xuejiang Guo[1,4,*], Feng Lu[2], Jing Dong[2], Yankai Xia[1,6], Yang Wen[1,2], Hao Wu[1,4], Honggang Li[7], Yong Zhu[2], Ping Ping[3], Xiangfeng Chen[3], Juncheng Dai[2], Yue Jiang[1,2], Shandong Pan[2], Peng Xu[8], Kailing Luo[9], Qiang Du[10], Bing Yao[11], Ming Liang[12], Yaoting Gui[13], Ning Weng[8], Hui Lu[3], Zhuqing Wang[3], Fengbin Zhang[14], Xiaobin Zhu[3], Xiaoyu Yang[1,15], Zhou Zhang[16], Han Zhao[17], Chenliang Xiong[7], Hongxia Ma[2], Guangfu Jin[2], Feng Chen[2], Jianfeng Xu[18], Xinru Wang[1,6], Zuomin Zhou[1,4], Zi-Jiang Chen[17], Jiayin Liu[1,15], Hongbing Shen[1,2] & Jiahao Sha[1,4]

OXFORD
Human Molecular Genetics, 2015, Vol. 24, No. 5 1493–1503
doi: 10.1093/hmg/ddu557
Advance Access Publication Date: 30 October 2014
Original Article

ORIGINAL ARTICLE

Identification of seven genes essential for male fertility through a genome-wide association study of non-obstructive azoospermia and RNA interference-mediated large-scale functional screening in *Drosophila*

Jun Yu[1,2,†], Hao Wu[1,2,†], Yang Wen[1,4,†], Yujuan Liu[1,2,†], Tao Zhou[1,2], Bixian Ni[1,4], Yuan Lin[1,4], Jing Dong[1,4], Zuomin Zhou[1,2], Zhibin Hu[1,4,*], Xuejiang Guo[1,2,*], Jiahao Sha[1,2,*], and Chao Tong[3,*]

NATURE GENETICS VOLUME 44 | NUMBER 2 | FEBRUARY 2012

nature genetics

A genome-wide association study in Chinese men identifies three risk loci for non-obstructive azoospermia

Zhibin Hu[1,2,11], Yankai Xia[1,3,11], Xuejiang Guo[1,4,11], Juncheng Dai[2], HongGang Li[5], Hongliang Hu[6,7], Yue Jiang[2], Feng Lu[2], Yibo Wu[1,4], Xiaoyu Yang[1,8], Huizhang Li[2], Bing Yao[9], Chuncheng Lu[3], Chenliang Xiong[5], Zheng Li[6,7], Yaoting Gui[10], Jiayin Liu[1,8], Zuomin Zhou[1,4], Hongbing Shen[1,2], Xinru Wang[1,3] & Jiahao Sha[1,4]

OXFORD
Human Molecular Genetics, 2015, Vol. 24, No. 19 5628–5636
doi: 10.1093/hmg/ddv257
Advance Access Publication Date: 21 July 2015
Association Studies Article

ASSOCIATION STUDIES ARTICLE

Low-frequency germline variants across 6p22.2–6p21.33 are associated with non-obstructive azoospermia in Han Chinese men

Bixian Ni[1,4,†], Yuan Lin[1,4,†], Liangdan Sun[6,7,†], Meng Zhu[4,†], Zheng Li[8], Hui Wang[4], Jun Yu[1,2], Xuejiang Guo[1,2], Xianbo Zuo[6,7], Jing Dong[4], Yankai Xia[1,5], Yang Wen[1,4], Hao Wu[1,2], Honggang Li[9], Yong Zhu[8], Ping Ping[8], Xiangfeng Chen[8], Juncheng Dai[4], Yue Jiang[1,4], Peng Xu[10], Qiang Du[11], Bing Yao[12], Ning Weng[10], Hui Lu[8], Zhuqing Wang[8], Xiaobin Zhu[8], Xiaoyu Yang[1,3], Chenliang Xiong[9], Hongxia Ma[4], Guangfu Jin[4], Jianfeng Xu[13], Xinru Wang[1,5], Zuomin Zhou[1,2], Jiayin Liu[1,3], Xuejun Zhang[6,7], Donald F. Conrad[15,16], Zhibin Hu[1,4,14,*] and Jiahao Sha[1,2,*]

CNV STUDIES

OPEN ACCESS Freely available online

PLoS one

Copy Number Variants in Patients with Severe Oligozoospermia and Sertoli-Cell-Only Syndrome

Frank Tüttelmann[1]*, Manuela Simoni[2], Sabine Kliesch[3], Susanne Ledig[1], Bernd Dworniczak[1], Peter Wieacker[1], Albrecht Röpke[1]

Copy number variation associated with meiotic arrest in idiopathic male infertility

Stefanie Eggers, Ph.D.,[a] Kathleen D. DeBoer, Ph.D.,[b] Jocelyn van den Bergen, B.Sc.,[a] Lavinia Gordon, M.Sc.,[a] Stefan J. White, Ph.D.,[c] Duangporn Jamsai, Ph.D.,[b] Robert I. McLachlan, Ph.D.,[d,e] Andrew H. Sinclair, Ph.D.,[a]

Single nucleotide polymorphism array analysis in men with idiopathic azoospermia or oligoasthenozoospermia syndrome

Anne Frühmesser, Ph.D.,[a] Peter H. Vogt, Ph.D.,[b] Jutta Zimmer,[b] Martina Witsch-Baumgartner, Ph.D.,[a] Christine Fauth, M.D.,[a] Johannes Zschocke, Ph.D., M.D.,[a] Germar-Michael Pinggera, M.D.,[c] and Dieter Kotzot, M.D.[a]

Gene 506 (2012) 248–252

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Genome-wide screening of severe male factor infertile patients using BAC-array comparative genomic hybridization (CGH)☆

Seung-Hun Song [a,1], Sung Han Shim [b,1], Jeong Kyoon Bang [a], Ji Eun Park [b], Se Ra Sung [b], Dong Hyun Cha [c,*]

OPEN ACCESS Freely available online

PLOS | GENETICS

Human Spermatogenic Failure Purges Deleterious Mutation Load from the Autosomes and Both Sex Chromosomes, including the Gene *DMRT1*

Alexandra M. Lopes[1☯*], Kenneth I. Aston[2☯], Emma Thompson[3], Filipa Carvalho[4], João Gonçalves[5], Ni Huang[6], Rune Matthiesen[1], Michiel J. Noordam[6], Inés Quintela[7], Avinash Ramu[6], Catarina Seabra[1], Amy B. Wilfert[6], Juncheng Dai[8], Jonathan M. Downie[9], Susana Fernandes[4], Xuejiang Guo[10,11], Jiahao Sha[10,11], António Amorim[1,12], Alberto Barros[4,13], Angel Carracedo[7,14], Zhibin Hu[8,10], Matthew E. Hurles[15], Sergey Moskovtsev[16,17], Carole Ober[3,18], Darius A. Paduch[19], Joshua D. Schiffman[9,20,21], Peter N. Schlegel[19], Mário Sousa[22], Douglas T. Carrell[2,23,24], Donald F. Conrad[6,25*]

ANDROLOGY

American Society of Andrology E·A·A

Explore this journal >

Original Article

Copy number variations in testicular maturation arrest

A. Halder ✉, P. Kumar, M. Jain, V. K. Iyer

OPEN ACCESS Freely available online

PLOS | ONE

High Resolution X Chromosome-Specific Array-CGH Detects New CNVs in Infertile Males

Csilla Krausz[1,2*], Claudia Giachini[1], Deborah Lo Giacco[2,3], Fabrice Daguin[1], Chiara Chianese[1], Elisabet Ars[3], Eduard Ruiz-Castane[2], Gianni Forti[4], Elena Rossi[5]

Human Reproduction, Vol.27, No.3 pp. 921–929, 2012
Advanced Access publication on January 11, 2012 doi:10.1093/humrep/der440

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Array comparative genomic hybridization in male infertility

K. Stouffs[1,2,*], D. Vandermaelen[1,2], A. Massart[1,2], B. Menten[3], S. Vergult[3], H. Tournaye[4,5], and W. Lissens[1,2]

Genomic and genetic variation in E2F transcription factor-1 in men with nonobstructive azoospermia

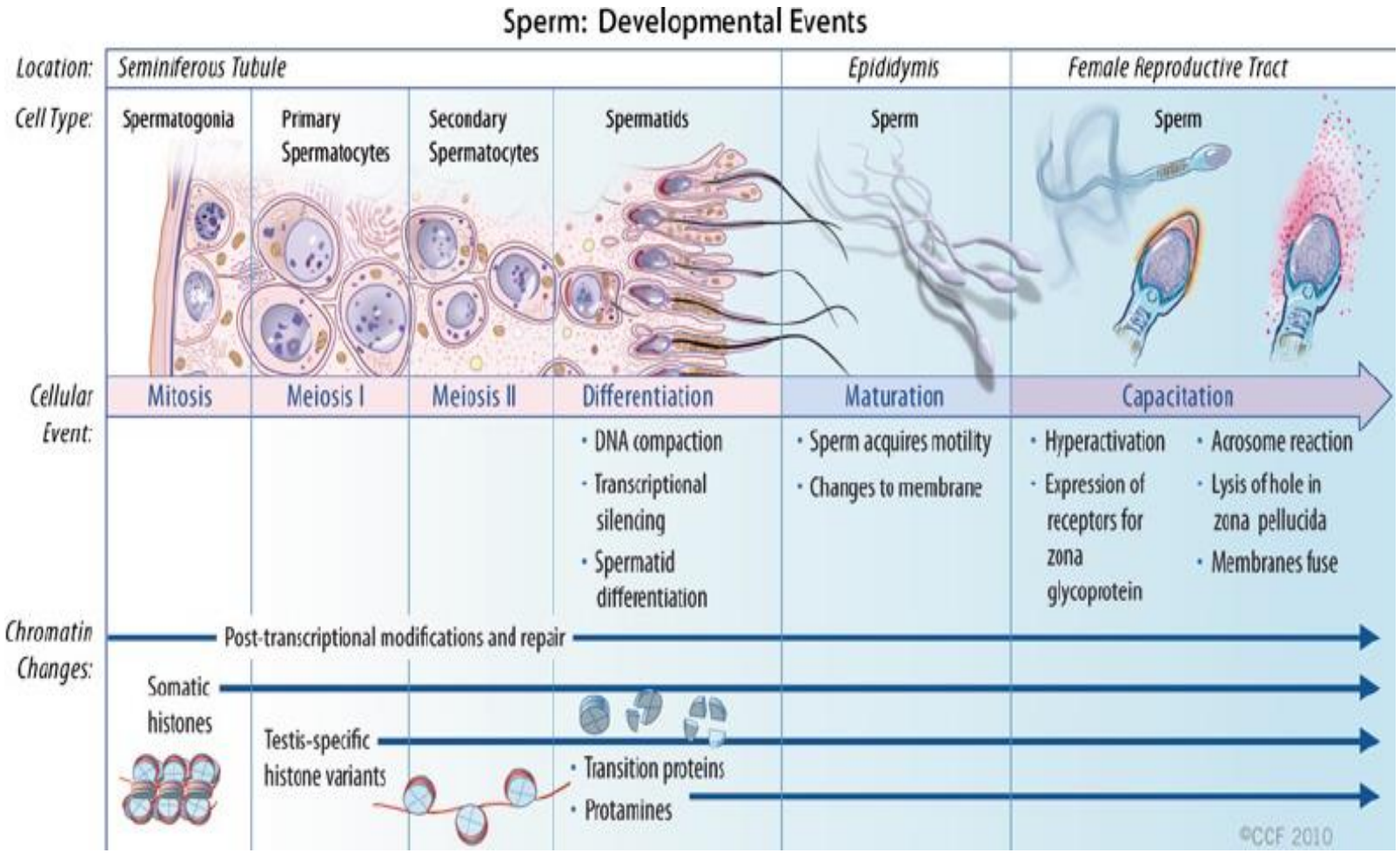
Carolina J. Jorgez, Ph.D.,[a,b] Nathan Wilken, B.S.,[a,b] Josephine B. Addai, B.S.,[a,b] Justin Newberg, Ph.D.,[c] Hima V. Vangapandu, M.S.,[b] Alexander W. Pastuszak, M.D., Ph.D.,[a,b] Sarmistha Mukherjee, Ph.D.,[b] Jill A. Rosenfeld, M.S.,[d] Larry I. Lipshultz, M.D.,[a,b] and Dolores J. Lamb, Ph.D.[a,b,c]

GMR

Copy number variations in spermatogenic failure patients with chromosomal abnormalities and unexplained azoospermia

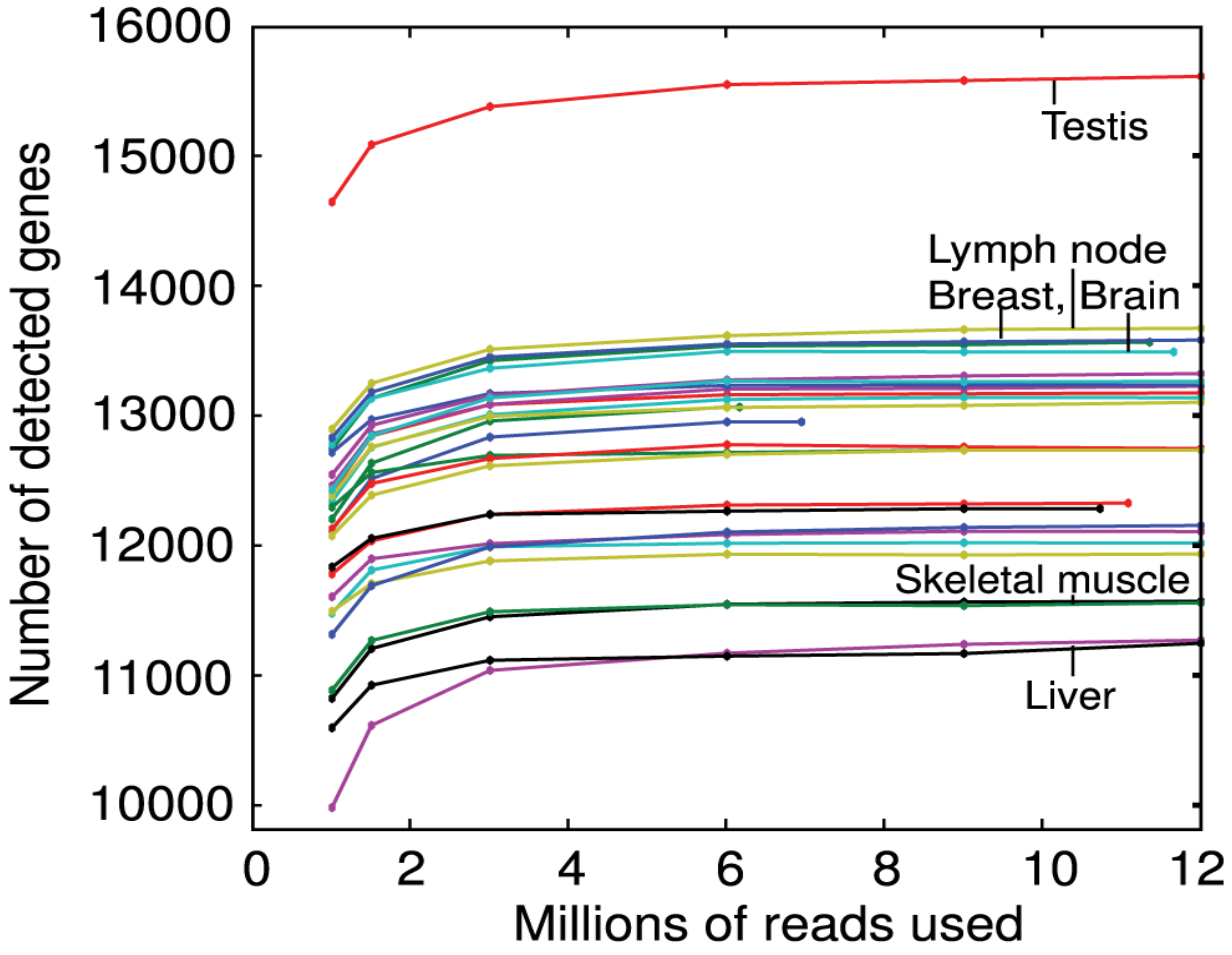
Y. Dong[1,2], Y. Pan[1], R. Wang[1], Z. Zhang[1], Q. Xi[1] and R.-Z. Liu[1]

COMPLEXITY OF SPERMATOGENESIS



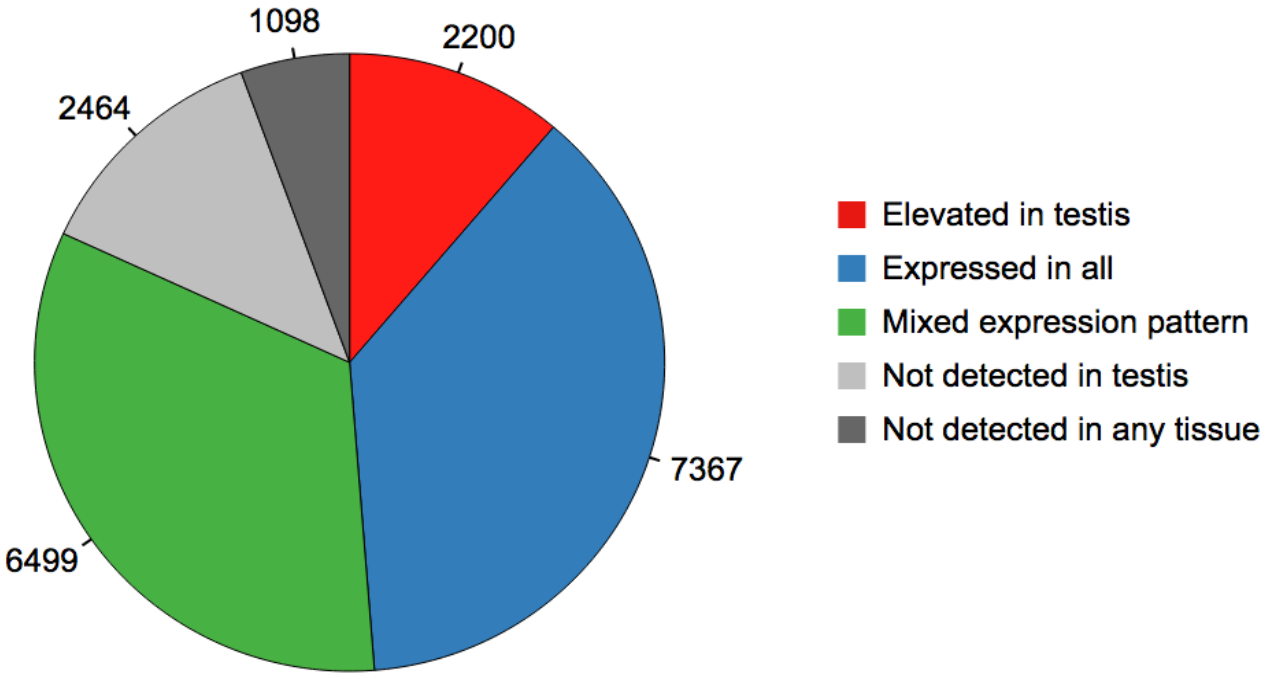
Rakesh Sharma and Ashok Agarwal, Sperm Chromatin: Biological and Clinical Applications in Male Infertility and Assisted Reproduction, 2011

84% OF ALL GENES ARE EXPRESSED IN THE TESTIS



Daniel Ramsköld et al. PLOS Computational Biology, December 11, 2009

82% OF ALL PROTEINS ARE EXPRESSED IN THE TESTIS



Human Protein Atlas

http://www.proteinatlas.org/humanproteome/testis

CHALLENGES

- Multitude of potential loci
- Genetically/phenotypically heterogeneous disease
- Limited sample sets
- Challenges of functional validation

SOLUTIONS

- **Collaboration**
- Whole genome approaches capable of detecting rare genomic variants
- Development of custom analytical tools
- Application of powerful tools for *in vitro* and *in vivo* validation

COLLABORATION:

MONASH University

UNIVERSITY OF COPENHAGEN

STANFORD SCHOOL OF MEDICINE

BROWN Alpert Medical School

UNIVERSITY OF WASHINGTON

THE TUREK CLINIC

McGill

Weill Cornell Medical College

THE UNIVERSITY OF CHICAGO

U.PORTO UNIVERSIDADE DO PORTO

Washington WASHINGTON·UNIVERSITY·IN·ST·LOUIS

UCSF University of California San Francisco

The University of Utah School of Medicine

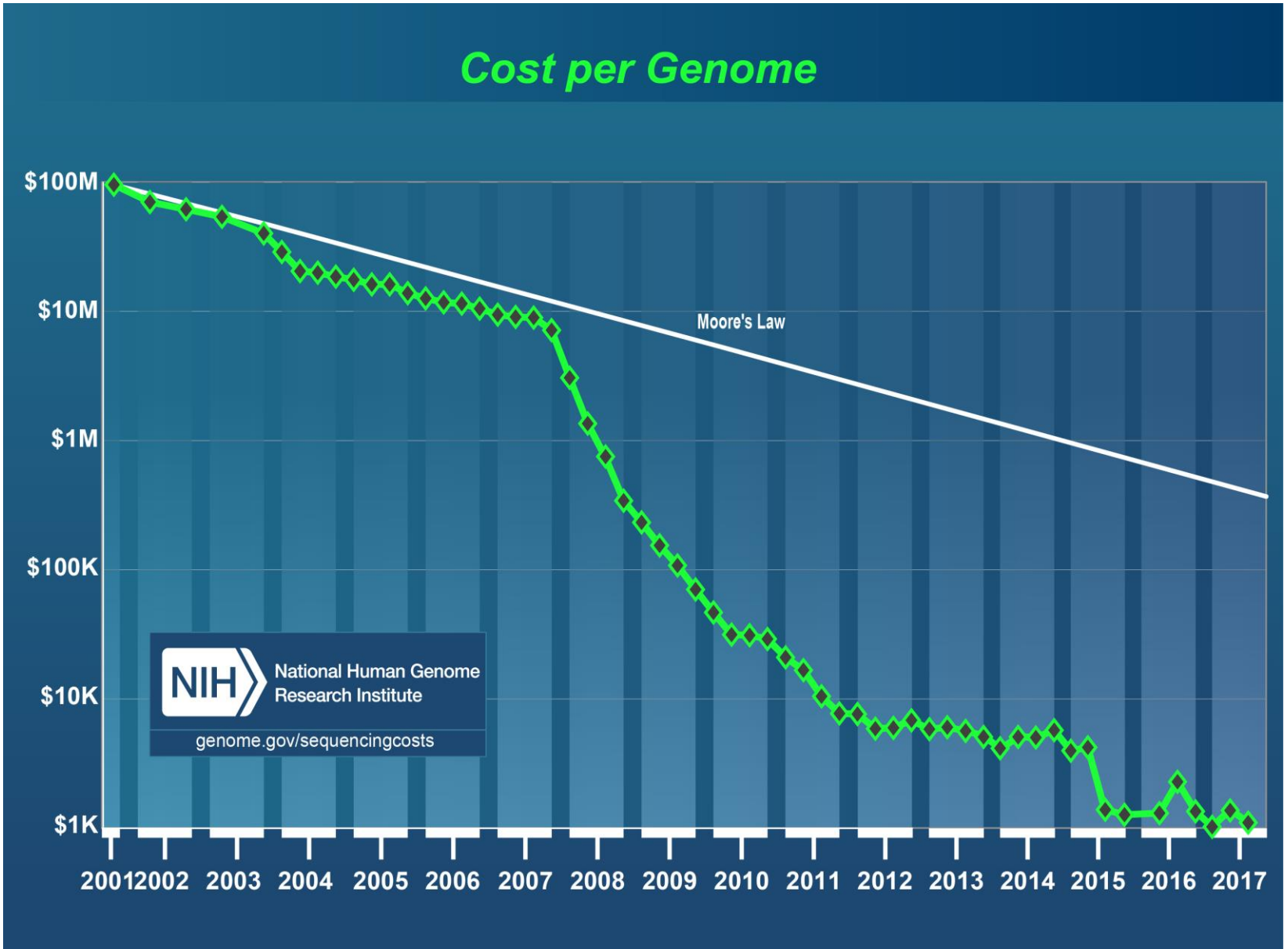
MOUNT SINAI HOSPITAL

TARTU ÜLIKOOL 1632 UNIVERSITAS TARTUENSIS

KU THE UNIVERSITY OF KANSAS

MEDICAL COLLEGE OF WISCONSIN

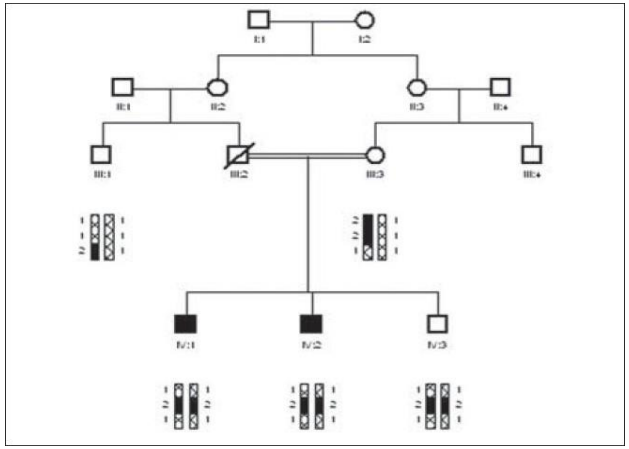
INCREASING ACCESSIBILITY TO LARGE-SCALE SEQUENCING:



GEMINI'S APPROACH

- Exome sequencing of 1000 NOA cases
- Identify likely variants
- Functional validation in cell lines, animal models, etc.

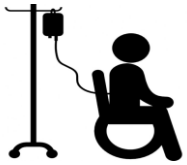
APPROACHES TO MAPPING DISEASE VARIANTS



LINKAGE ANALYSIS

Vs.

ASSOCIATION ANALYSIS



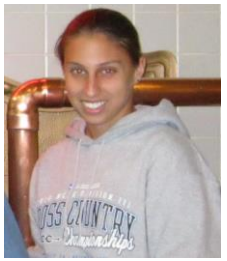
Vs.

N=1 ANALYSIS

N=1 ANALYSIS:

- What is the probability that a given genetic variant identified in an infertile man will be found in a healthy, fertile population?
- Analysis is conditional on the functional effects of the genotypes.
- PSAP=population sampling probability

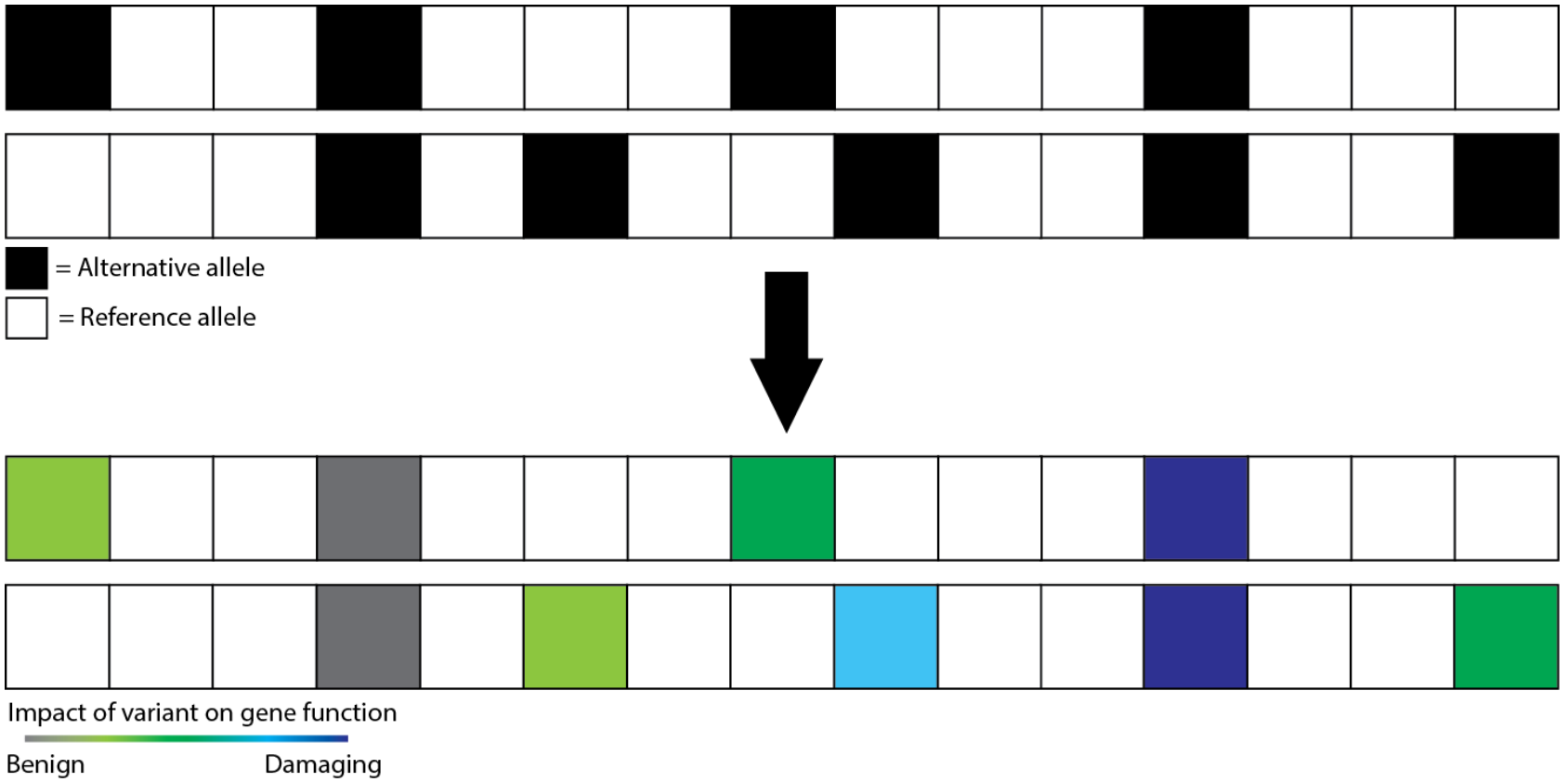
Wilfert, et al. Nature Genetics, 2016



Amy Wilfert

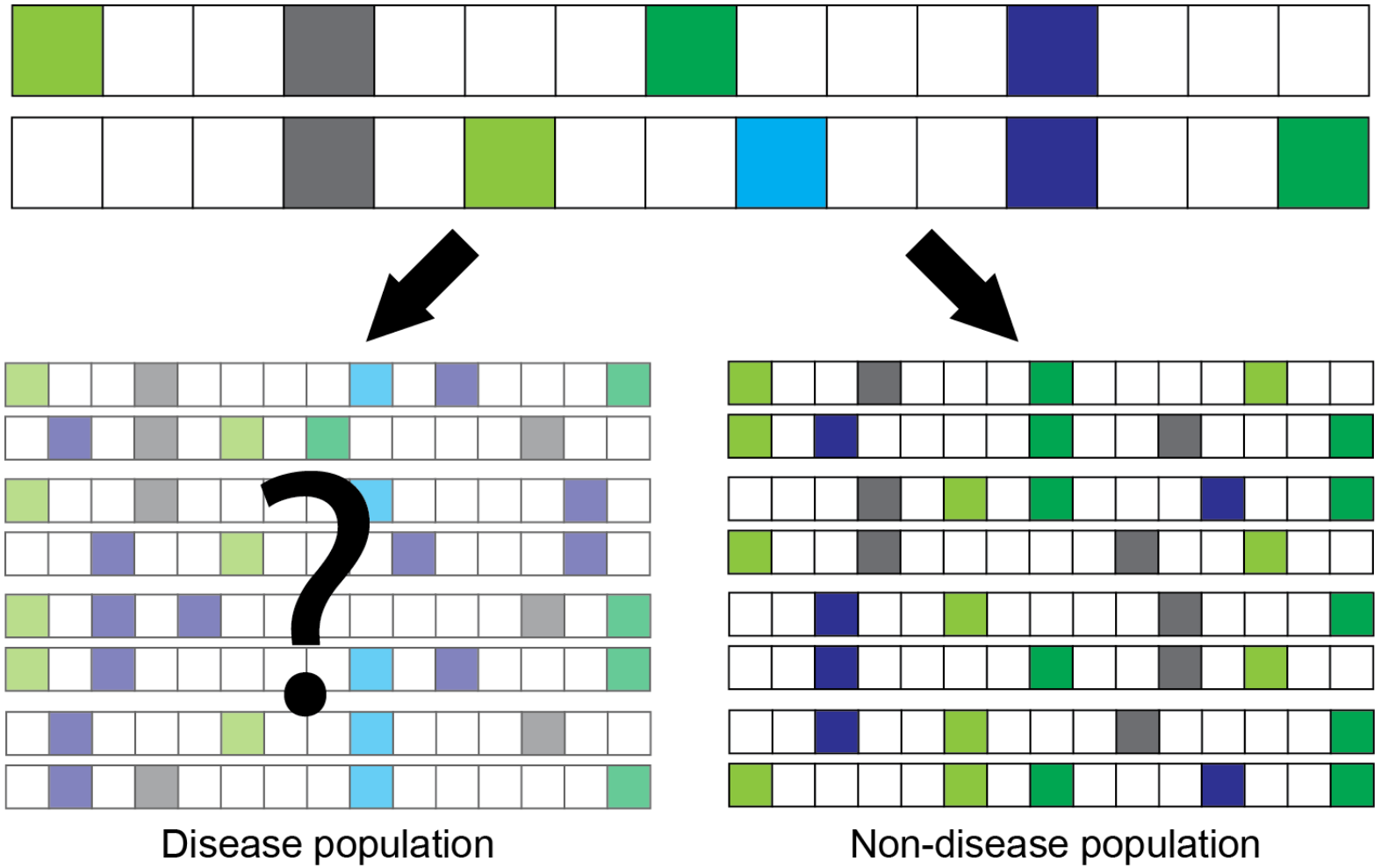
VARIANT ANNOTATION

Identifying variants that are damaging to gene function

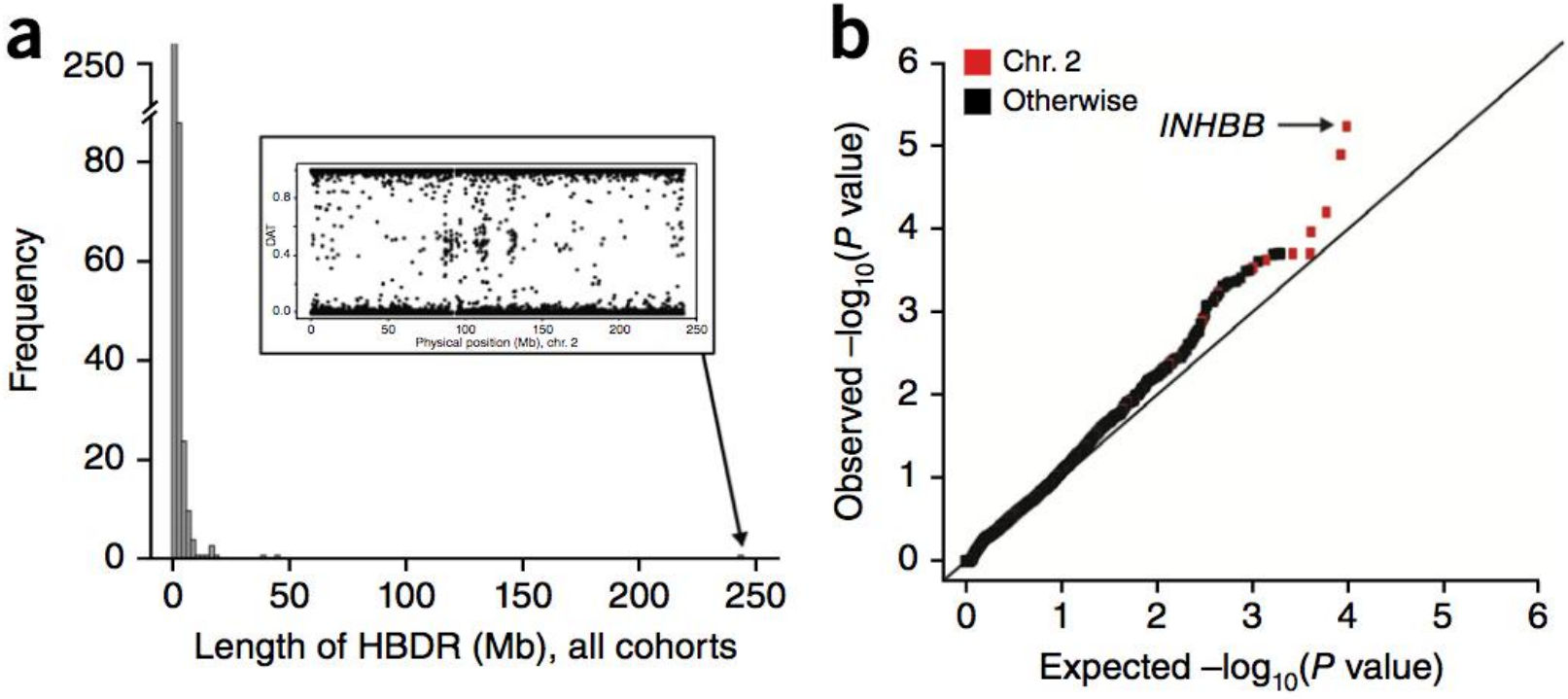


CADD (Kircher, et al. 2014 Nature Genetics)

SIGNIFICANCE TESTING



THE MOTIVATING CASE: 30 YR OLD NOA MAN WITH UPD2



Wilfert, et al. Nature Genetics, 2016

GENETICS RESULTS: HUMAN KNOCKOUTS IN GEMINI

- “Loss-of-function” mutations can be easily recognized (e.g. stop gains, splice mutations)
- Provide a clear expectation of functional impact
- Can be used to infer biological function, and drug targets
- 3,436 knockout genes reported to date (ExAC, deCODE, East London Genes project and HGMD)

Nature Reviews Genetics | Published online 2 May 2017; doi:10.1038/nrg.2017.35

“enormous potential of reverse genetics to expand the field of functional human genetics”

GEMINI SAMPLES

Sample collection ongoing

3650 men recruited (Nov 2017)

- 1642 cases
- 2008 controls

Phase I sequencing

Total, 890 samples:

506 analyzed

384 in analysis

Center	Cases	Controls
PRT	296	78
AUS	11	0
DEN	91	0
WashU	24	6
Total	422	84

OTHER KNOCKOUT NOA CASES

10 KO genes - novel candidates in testis biology/infertility

1. Function mostly unknown
2. No knockouts observed previously for **5 genes**
   (Not in all known 3436 knockout genes)

AXDND1 Highest in testis, Nothing known
MAGEB4 Highest in testis, published stoploss in Turkish azoospermia brothers
PNLDC1 Highest in testis, Processing of piRNAs
SPIDR DNA double strand break repair
ZNF512B MicroRNA regulation?

VALIDATION:

WHY VALIDATE?

Based on current GEMINI analysis

- Rare likely disease-causing mutations in 236 genes
- 92% of genes are case-specific

  Unlikely to find multiple carriers of mutations in these genes
- Validation screening of top genes in model organisms

FUNCTIONAL VALIDATION OF TOP GENES

Mouse

KO/CRISPR of 2 novel testis genes

GEMINI collaborator Moira O'Brian (Monash University, Australia)

Chlamydomonas

Potential ciliary gene *CCDC112* (Susan Dutcher; WashU)

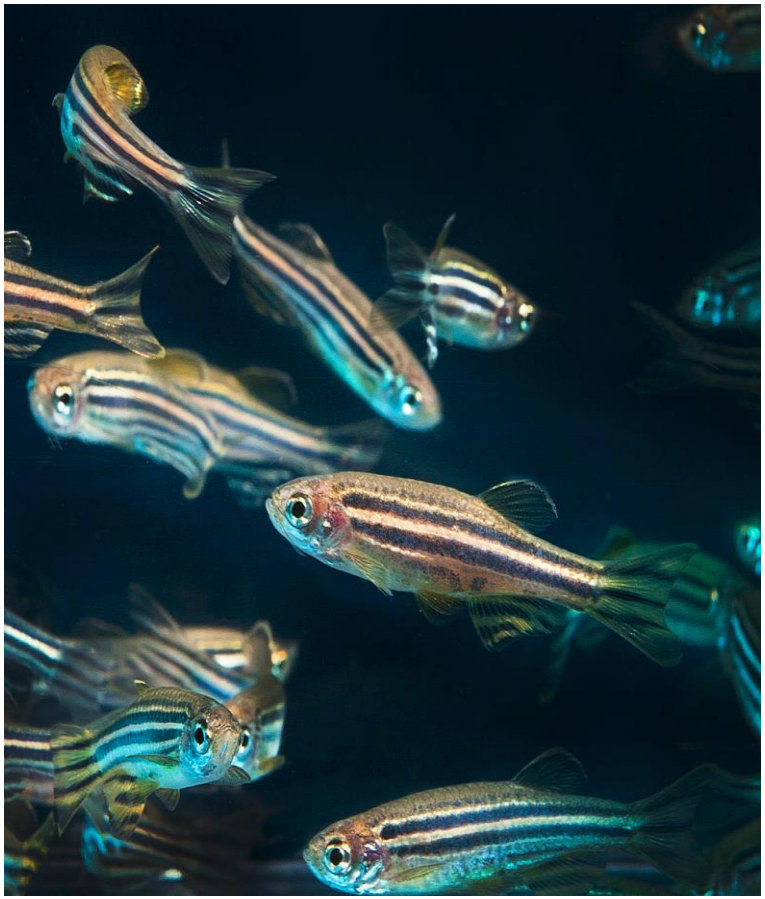
C. elegans

DNA double-strand break repair gene *RAD50* (Tim Schedl; WashU)

Drosophila

Screening via testis-specific RNAi (Conrad lab, WashU)

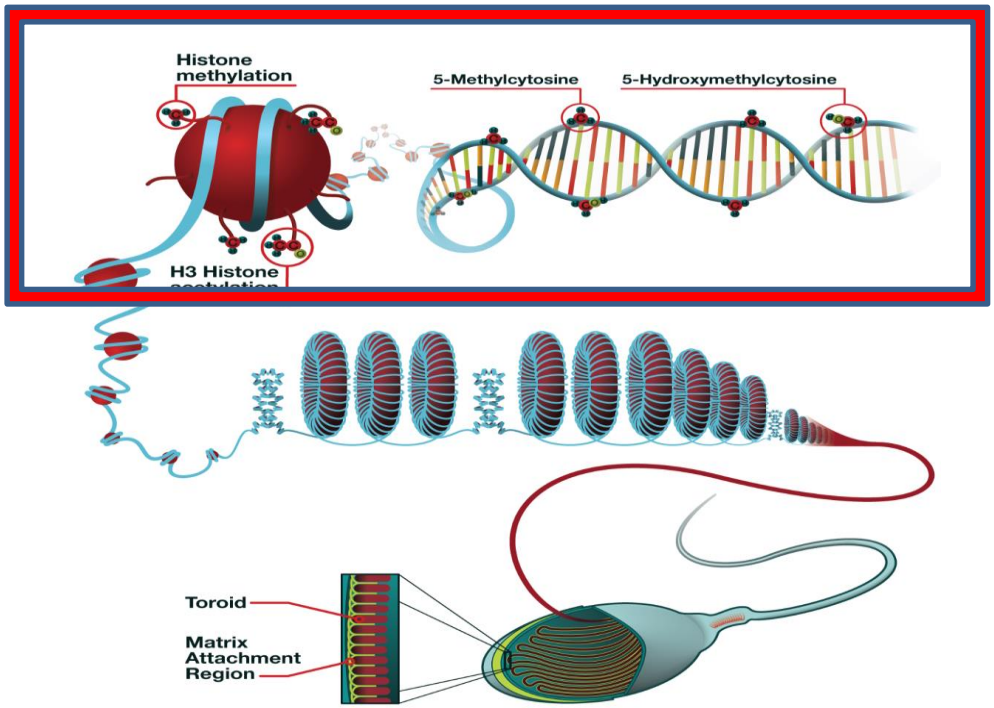
ZEBRAFISH AS A MODEL FOR NOA



SPERM EPIGENETICS

- Associations with male infertility
- What impacts sperm epigenetics?
  - Age
  - Smoking
- Effects on offspring?

SPERM PROTAMINATION AND EPIGENETICS



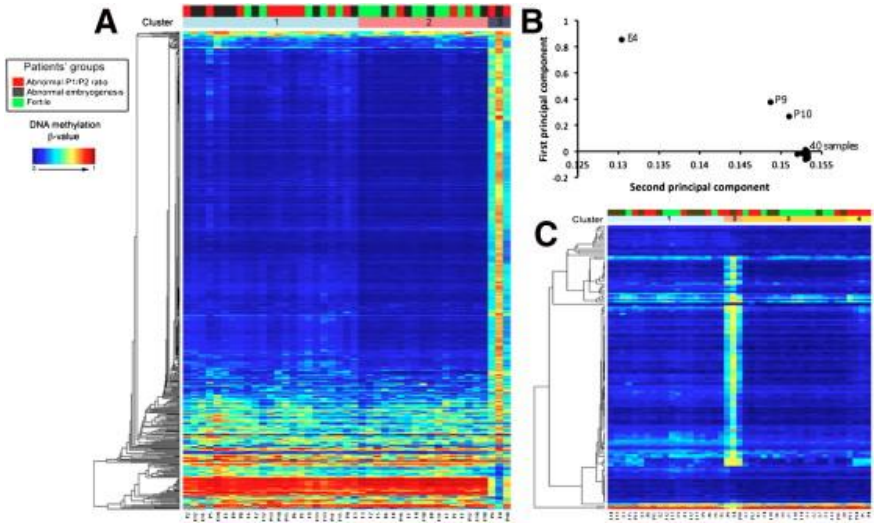
THE "POISED FOR EMBRYOGENESIS" SPERM EPIGENOME

- Most of the sperm genome (>90%) is silenced by protamine replacement of histones.
- Key embryogenesis genes are not protaminated, and are epigenetically "poised" for rapid activation in embryogenesis.
- These marks are largely set in the spermatogonial stem cells.
- This unique poising is conserved in nature (likely means its very important).
- The pattern suggests a role of sperm contributing to embryogenesis.

From Hammoud et al., 2009; Carrell et al., 2013

FREQUENCY OF ABNORMAL METHYLATION IN PATIENTS WITH POOR IVF EMBRYOGENESIS HISTORY

- Association testing across all loci:
  - 6.7% of loci were abnormally methylated (Bonferroni $p < 0.01$)
- Imprinted loci:
  - 43.6% of DMR CpGs were abnormally methylated



Aston et al. Fertility & Sterility **97**, 285-292 (2012)

INITIAL STUDIES

Aberrant sperm DNA methylation predicts male fertility status and embryo quality

Kenneth I. Aston, Ph.D.,[a] Philip J. Uren, Ph.D.,[b] Timothy G. Jenkins, Ph.D.,[a] Alan Horsager, Ph.D.,[c] Bradley R. Cairns, Ph.D.,[d,e] Andrew D. Smith, Ph.D.,[b] and Douglas T. Carrell, Ph.D.[a,f]

[a] Department of Surgery, University of Utah Andrology and IVF Laboratories, University of Utah School of Medicine, Salt Lake City, Utah; [b] Molecular and Computational Biology, University of Southern California, Los Angeles, California; [c] Episona, Inc, Glendale, California; [d] Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, Utah; [e] Howard Hughes Medical Institute, Chevy Chase, Maryland; and [f] Department of Obstetrics and Gynecology and Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah

Objective: To evaluate whether male fertility status and/or embryo quality during in vitro fertilization (IVF) therapy can be predicted based on genomewide sperm deoxyribonucleic acid (DNA) methylation patterns.
Design: Retrospective cohort study.
Setting: University-based fertility center.
Patient(s): Participants were 127 men undergoing IVF treatment (where any major female factor cause of infertility had been ruled out), and 54 normozoospermic, fertile men. The IVF patients were stratified into 2 groups: patients who had generally good embryogenesis and a positive pregnancy (n = 55), and patients with generally poor embryogenesis (n = 72; 42 positive and 30 negative pregnancies) after IVF.
Intervention(s): Genomewide sperm DNA methylation analysis was performed to measure methylation at >485,000 sites across the genome.
Main Outcome Measure(s): A comparison was made of DNA methylation patterns of IVF patients vs. normozoospermic, fertile men.
Result(s): Predictive models proved to be highly accurate in classifying male fertility status (fertile or infertile), with 82% sensitivity, and 99% positive predictive value. Hierarchic clustering identified clusters enriched for IVF patient samples and for poor-quality–embryo samples. Models built to identify samples within these groups, from neat samples, achieved positive predictive value ≥94% while identifying >one fifth of all IVF patient and poor-quality–embryo samples in each case. Using density gradient prepared samples, the same approach recovered 46% of poor-quality–embryo samples with no false positives.
Conclusion(s): Sperm DNA methylation patterns differ significantly and consistently for infertile vs. fertile, normozoospermic men. In addition, DNA methylation patterns may be predictive of embryo quality during IVF. (Fertil Steril® 2015;104:1388–97. ©2015 by American Society for Reproductive Medicine.)
Key Words: Sperm DNA, DNA methylation, IVF outcome, embryo, genomewide, microarray, male infertility

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertilitytforum.com/astonk-sperm-dna-methylation-infertility/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received June 1, 2015; revised July 29, 2015; accepted August 18, 2015; published online September 8, 2015.
K.I.A. has a patent in preparation entitled Methods of Identifying Male Fertility Status and Embryo Quality, P.J.U. has a patent in preparation entitled Methods of Identifying Male Fertility Status and Embryo Quality, T.G.J. has a patent in preparation entitled Methods of Identifying Male Fertility Status and Embryo Quality, A.H. is a shareholder for Episona, Inc and is licensing this technology with Episona, Inc. B.R.C. has a patent in preparation entitled Methods of Identifying Male Fertility Status and Embryo Quality, A.D.S. has a patent in preparation entitled Methods of Identifying Male Fertility Status and Embryo Quality; and is a shareholder for Episona, Inc. D.T.C. is a shareholder for Episona, Inc.
Reprint requests: Douglas T. Carrell, Ph.D., Division of Urology-Andrology/IVF Laboratories, University of Utah School of Medicine, Andrology, 675 S Arapeen Dr, Ste #205, Salt Lake City, Utah 84108 (E-mail: douglas.carrell@hsc.utah.edu).

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http://dx.doi.org/10.1016/j.fertnstert.2015.08.019

The mainstay of male infertility diagnosis is the standard semen analysis. With the exception of modification of criteria for morphologic grading, semen analysis has changed very little over the past several decades. Numerous studies have evaluated the prognostic value of the various semen parameters evaluated by the standard analysis (1–3). Except for severely diminished sperm count or motility, the predictive value of semen analysis for

Decreased fecundity and sperm DNA methylation patterns

Timothy G. Jenkins, Ph.D.,[a] Kenneth I. Aston, Ph.D.,[a] Tyson D. Meyer, B.S.,[a] James M. Hotaling, M.D., M.S.,[a] Monis B. Shamsi, Ph.D.,[a] Erica B. Johnstone, M.D.,[b] Kyley J. Cox, M.P.H.,[c] Joseph B. Stanford, M.D., M.S.P.H.,[c] Christina A. Porucznik, Ph.D., M.S.P.H.,[c] and Douglas T. Carrell, Ph.D.[a,b,c,d]

[a] Division of Urology, Department of Surgery, [b] Department of Obstetrics and Gynecology, [c] Department of Family and Preventive Medicine, and [d] Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah

Objective: To evaluate the relationship between epigenetic patterns in sperm and fecundity.
Design: Prospective study.
Setting: Academic andrology and in vitro fertilization laboratory.
Patient(s): Twenty-seven semen samples from couples who conceived within 2 months of attempting a pregnancy and 29 semen samples from couples unable to achieve a pregnancy within 12 months.
Intervention(s): None.
Main Outcome Measure(s): Genomewide assessment of differential sperm DNA methylation and standard semen analysis.
Result(s): We analyzed DNA methylation alterations associated with fecundity in 124 semen samples, and identified regions of interest in 27 semen samples from couples who conceived within 2 months of attempting a pregnancy and a total of 29 semen samples from couples who were unable to achieve a pregnancy within 12 months. No differences in sperm count, sperm morphology, or semen volume were observed between the patients achieving a pregnancy within 2 months of study time and those not obtaining a pregnancy within 12 months. However, using data from the human methylation 450k array analysis we did identify two genomic regions with statistically significantly decreased (false discovery rate <0.01) methylation and three genomic regions with statistically significantly increased methylation in the failure-to-conceive group. The only two sites where decreased methylation was associated with reduced fecundity are at closely related genes known to be expressed in sperm, *HSPA1L* and *HSPA1B*.
Conclusion(s): Our data suggest that there are genomic loci where DNA methylation alterations are associated with decreased fecundity. We have thus identified candidate loci for future study to verify these results and investigate the causative or contributory relationship between altered sperm methylation and decreased fecundity. (Fertil Steril® 2016;105:51–7. ©2016 by American Society for Reproductive Medicine.)
Key Words: DNA methylation, epigenetics, HSP, male infertility, sperm

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertilitytforum.com/jenkinst-sperm-epigenetics-fecundity/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Decreased fecundity is a complex disease with many subtle associations with genetic, biologic, and lifestyle factors that contribute to the disease (1–6). This complexity is further compounded by the fact that this disease ultimately affects a couple, such that the etiology of reproductive dysfunction can be found in the female or male alone, or potentially in both partners. The frequency of infertility is varied worldwide (peaking at approximately 30% of couples in some regions), but is thought to impact an average of 8%–12% of all reproductive-aged couples globally (7–10). There are many known causes of infertility or subfertility, but often the etiology of individual cases cannot be identified.

A common source of subfertility or infertility in human couples is male subfertility or decreased fecundity. It is believed that male reproductive dysfunction is an independent cause of reduced fecundity in approximately 30% of subfertile couples (11). In another 20% of couples, abnormal male reproductive function contributes to a couple's inability to conceive but is not independently responsible (11). What constitutes male subfertility is difficult to elucidate, as there are many fertility-related diagnoses assigned to men based on abnormalities

Received May 11, 2015; revised and accepted September 10, 2015; published online October 9, 2015.
T.G.J. has a patent pending for methods of identifying male fertility status and embryo quality. K.I.A. has a patent pending for methods of identifying male fertility status and embryo quality. T.D.M. has nothing to disclose. J.M.H. has nothing to disclose. M.B.S. has nothing to disclose. E.B.J. has nothing to disclose. K.J.C. has nothing to disclose. J.B.S. has nothing to disclose. C.A.P. has nothing to disclose. D.T.C. is a stockholder in Episona, Inc., and has patents pending regarding sperm epigenetics, embryogenesis, aging, and fertility.
Supported in part by a grant from the National Institutes of Health (to D.T.C.).
Reprint requests: Douglas T. Carrell, Ph.D., 675 Arapeen Drive, Suite 205, Salt Lake City, Utah 84108 (E-mail: douglas.carrell@hsc.utah.edu).

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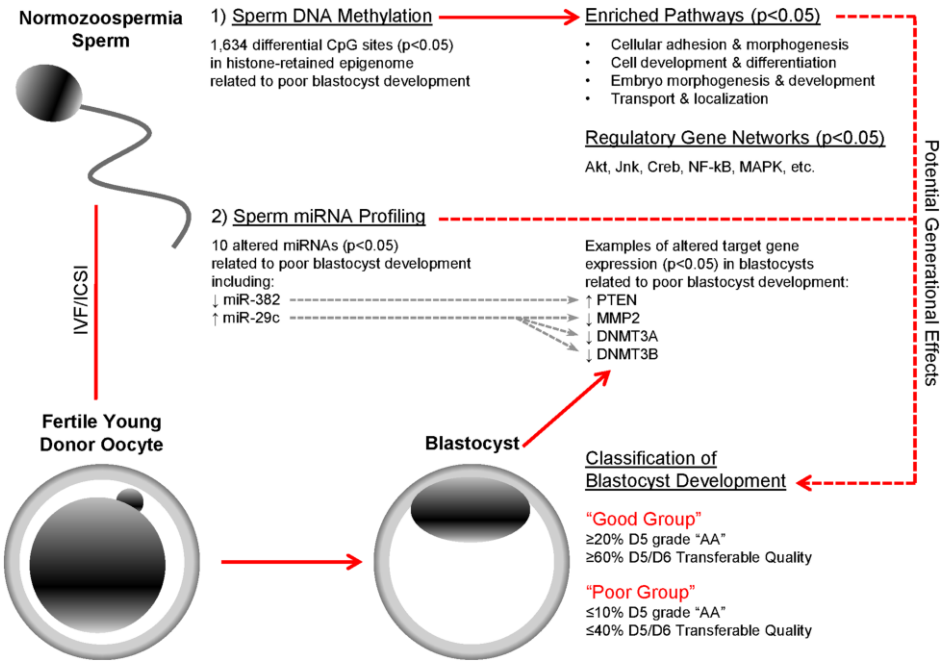
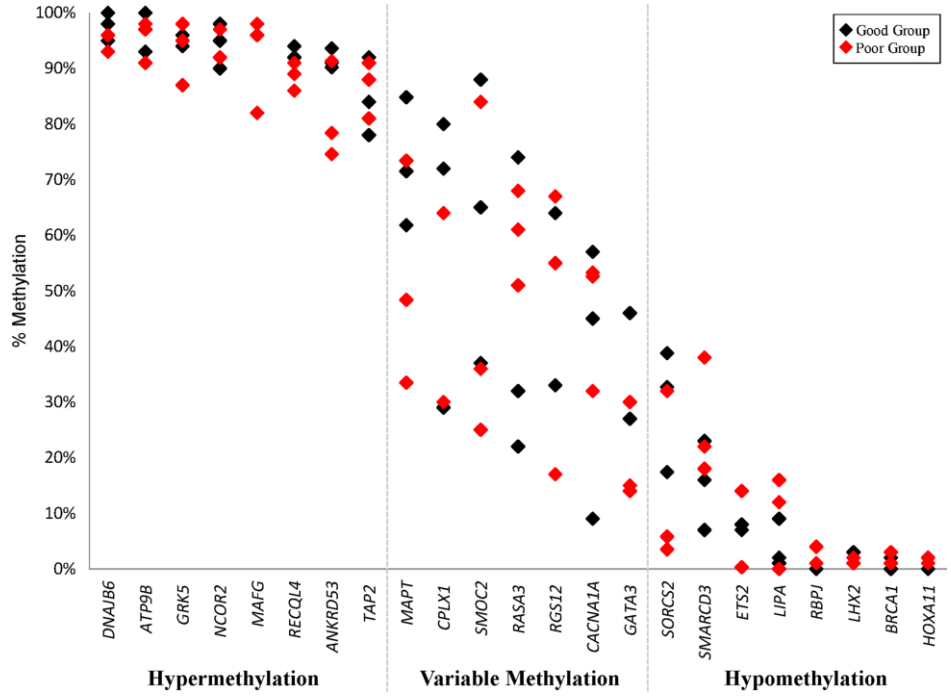
http://dx.doi.org/10.1016/j.fertnstert.2015.09.013



From: Alterations in the sperm histone-retained epigenome are associated with unexplained male factor infertility and poor blastocyst development in donor oocyte IVF cycles

Hum Reprod. 2017;32(12):2443-2455. doi:10.1093/humrep/dex317



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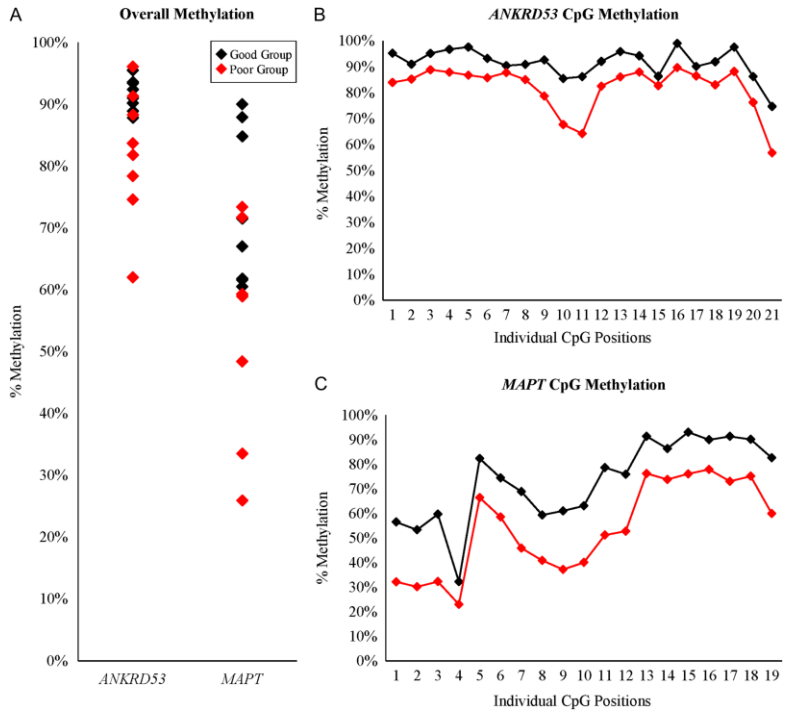

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SUPPORTING STUDIES

Example studies examining the correlation between global DNA methylation levels; columns indicate phenotypic associations identified in the study.

	Sperm Count	Morph. / Motil.	Fertility	Pregnancy Outcome	DNA fragmentation
Benchaib et al., Hum. Reprod. 2004	--	--	--	Yes	--
Houshdaran et al., PLoS ONE, 2007	Yes	Yes	Yes	--	--
Urdinguio et al., Hum. Reprod. 2015	--	--	Yes	--	--
Montjean et al., Andrology, 2015	Yes	Yes	--	--	Yes

Other studies, particularly more recent ones, have looked at epigenetic disruptions at specific genes. The focus is often on imprinted loci.

Study	Loci	Semen params.	Fertility	Embryo dev. / preg. / miscarriage
Marques et al., Mol. Hum. Reprod., 2008	H19, MEST, IGF2	Yes		Yes
Wu et al., PLoS ONE, 2010	MTHFR	Yes	Yes	
Hammoud et al., Fertil. Steril., 2010	LIT1, MEST, SNRPN, PLAGL1, PEG3, H19, and IGF2	Yes	Yes	
El Hajj et al., Sex Dev., 2011	H19, GTL2, LIT1, MEST, NESPAS, PEG3, SNRPN; ALU, LINE1		Yes	Yes
Ankolkar et al., Fertil. Steril., 2012	H19			Yes
Xu et al., Andrologia, 2016	MEST, GNAS, H19, FAM50B, LINE-1, P16	Yes	Yes	
Poplinski 2010 (Int. J. Andro.)	IGF2/H19 ICR1, MEST	Yes	Yes	
Urdinguio et al., Hum. Reprod. 2015	ALU repeats, 2752 CpGs (~1800 genes; ~60 imprinted)		Yes	
Kuhtz et al., Epigenetics, 2014	GTL2			Yes
Xu et al., Biol. Reprod., 2013	Pebp1	Yes		

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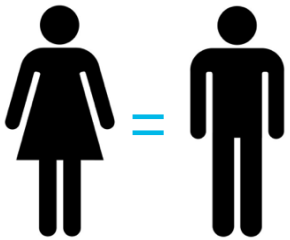
Kuhtz et al., Epigenetics, 2014	GTL2			Yes
Xu et al., Biol. Reprod., 2013	Pebp1	Yes		

Conclusions:

2 commercial assays
Growing rapidly
Expensive ($450 US)
Good Predictive Power
Likely to become standard

Male infertility



1 IN 10 COUPLES ARE INFERTILE[1]

WOMEN AND MEN ARE **EQUALLY AFFECTED**

… THE SEMEN ANALYSIS (STANDARD OF CARE) **PREDICTS MALE INFERTILITY VERY POORLY**

IUI IVF

15%

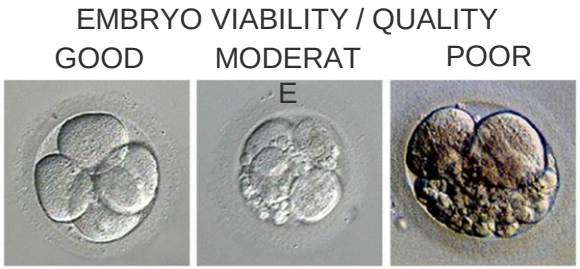
the sensitivity of the semen analysis for predicting infertility[2]

50%

of IVF treatment cycles fail, even when using IVF-ICSI[4]

22%

of infertility is of unknown cause (unexplained infertility)[3]



Summary

Semen Analysis (based on concentration threshold of 13.5 X 10^6 / ml, the best performing parameter threshold in this study[2].)	**14.8%** **SENSITIVITY** Percentage of suspected infertile men classified as infertile	**96.1%** **SPECIFICITY** Percentage of known fertile men identified as fertile
DNA Methylation Profile for Fertility	**84.3%**	**92.1%**
DNA Methylation Profile for embryo quality	**50.0%**	**94.0%**

Affected genes show function in sperm adhesion, chemotaxis and acrosome reaction. Functional defects likely to be missed by traditional semen analysis. Provides information to guide treatment.

HERITABILITY OF ENVIRONMENTAL EXPOSURES

- **Overkalix Sweden Study: Grandsons of pre-pubertal boys exposed to famine periods lived longer than those exposed to feast periods. When controlled for socioeconomic factors, difference was 32 years.**
- ALSPAC Study (England): Smoking during prepubertal period resulted in increased risk of obesity in offspring.
- Dutch Famine effects on pregnant mothers in early pregnancy resulted in lower methylation of IGF gene in offspring 60 years later.
- Agouti Mouse Study: Pregnant agouti mice fed vitamin B.
- Fruitfly exposure to geldanamycin causes bristly growths on eyes of offspring for many generations.

SPERM EPIGENETICS AND ENVIRONMENT

Study	Organism	Insult	Sperm Epigenome impact	Phenotype impact
Manikkam et al., PLoS ONE, 2013	*Mus musculus*	Endocrine disrupters (plastics) during primordial germ cell dev.	197 Diff. methylated sperm DNA regions	Pubertal abnormalities, testis disease, obesity, ovarian disease
Dong et al., 2016	*Homo sapiens*	Cigarette Smoking	Hypomethylation of H19 ICR	Infertility, oligozoospermia, asthenozoospermia, teratozoospermia
Skinner et al., BMC Med., 2013	*Rattus norvegicus*	Dichlorodiphenyltrichloroethane (DDT)	F3 generation sperm epimutations; genes associated with DMRs previously shown to be associated with obesity	F3 generation (great grand-offspring) had over 50% of males and females develop obesity.
Tsaprouni et al., 2014	*Homo sapiens*	Cigarette smoking		
Xu et al., Biol. Reprod., 2013	*Mus musculus*	Cigarette smoking	Pebp1 diff. methylation	Not assessed
Miao et al., Andrology, 2014	*Homo sapiens*	Bisphenol A (BPA) exposure.	Aberrant LINE1 repeat sperm methylation	Not assessed
Susiarjo et al., Endocrin. 2015	*Mus musculus*	Bisphenol A (BPA) exposure.	overexpression of the imprinted Igf2 gene; increased DNA methylation of Igf2 ICR.	higher body fat and perturbed glucose homeostasis in F1 and F2 male offspring

	Org.	Insult	Sperm Epigenome impact	Phenotype impact
Donkin et al., Cell Mat., 2015	*Homo sapiens*	Gastric bypass-induced weight loss	Genes involved in regulation of appetite and weight, including FTO (also implicated in male infertility)	Rapid and extreme weight loss
Denham et al., Epigenomics, 2015	*Homo sapiens*	Exercise intervention	Global changes in sperm DNA methylation; inc. genes related to schizophrenia and Parkinson's disease	Not reported
Palmer et al., Am. J. Physiol. Endocrinol. Metab., 2012	*Mus musculus*	Diet and exercise changes	Not assessed	improved sperm motility, morphology; reduced sperm DNA damage, reactive oxygen species; increased sperm binding

RISK FACTORS:

- Biological Factors
  - **Aging**
  - Obesity
  - Diet
  - Cancer
- Environmental Exposures
  - Smoking
  - Alcohol
  - Cancer Therapies
  - Medications
  - Air Pollution
  - Socio-economic stress
  - Toxic waste exposure

OPEN ACCESS Freely available online

PLOS | GENETICS

Age-Associated Sperm DNA Methylation Alterations: Possible Implications in Offspring Disease Susceptibility

Timothy G. Jenkins[1], Kenneth I. Aston[1], Christian Pflueger[2], Bradley R. Cairns[2,3]*, Douglas T. Carrell[1,4,5]*

1 Andrology and IVF Laboratories, Department of Surgery, University of Utah School of Medicine, Salt Lake City, Utah, United States of America, **2** Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, Utah, United States of America, **3** Howard Hughes Medical Institute, Chevy Chase, Maryland, United States of America, **4** Department of Genetics, University of Utah School of Medicine, Salt Lake City, Utah, United States of America, **5** Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, Utah, United States of America

Abstract

Recent evidence demonstrates a role for paternal aging on offspring disease susceptibility. It is well established that various neuropsychiatric disorders (schizophrenia, autism, etc.), trinucleotide expansion associated diseases (myotonic dystrophy, Huntington's, etc.) and even some forms of cancer have increased incidence in the offspring of older fathers. Despite strong epidemiological evidence that these alterations are more common in offspring sired by older fathers, in most cases the mechanisms that drive these processes are unclear. However, it is commonly believed that epigenetics, and specifically DNA methylation alterations, likely play a role. In this study we have investigated the impact of aging on DNA methylation in mature human sperm. Using a methylation array approach we evaluated changes to sperm DNA methylation patterns in 17 fertile donors by comparing the sperm methylome of 2 samples collected from each individual 9–19 years apart. With this design we have identified 139 regions that are significantly and consistently hypomethylated with age and 8 regions that are significantly hypermethylated with age. A representative subset of these alterations have been confirmed in an independent cohort. A total of 117 genes are associated with these regions of methylation alterations (promoter or gene body). Intriguingly, a portion of the age-related changes in sperm DNA methylation are located at genes previously associated with schizophrenia and bipolar disorder. While our data does not establish a causative relationship, it does raise the possibility that the age-associated methylation of the candidate genes that we observe in sperm might contribute to the increased incidence of neuropsychiatric and other disorders in the offspring of older males. However, further study is required to determine whether, and to what extent, a causative relationship exists.

Citation: Jenkins TG, Aston KI, Pflueger C, Cairns BR, Carrell DT (2014) Age-Associated Sperm DNA Methylation Alterations: Possible Implications in Offspring Disease Susceptibility. PLoS Genet 10(7): e1004458. doi:10.1371/journal.pgen.1004458

Editor: John M. Greally, Albert Einstein College of Medicine, United States of America

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Funding: An internal University of Utah small grant from the "University of Utah Center on Aging" was used for this study. Additionally, clinical funds were used for this study. No outside grant agency funds were applied. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: Brad.Cairns@hci.utah.edu (BRC); douglas.carrell@hsc.utah.edu (DTC)

Introduction

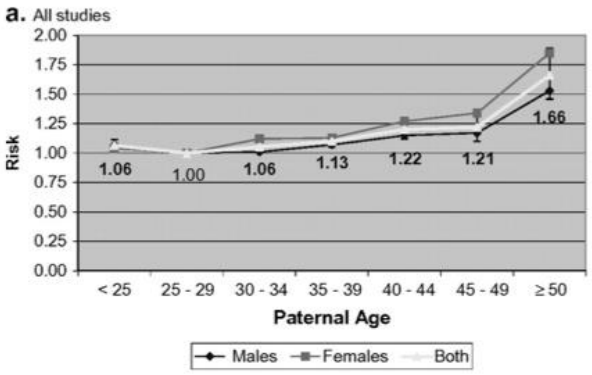
The effects of advanced paternal age have only recently become of interest to the scientific community as a whole. This interest has likely arisen as a result of recent studies that suggest an association with increased incidence of diseases and abnormalities in the offspring of older fathers. Specifically, offspring sired by older fathers have been shown to have increased incidence of neuropsychiatric disorders (autism, bipolar disorder, schizophrenia, etc.) [1–3], trinucleotide repeat associated diseases (myotonic dystrophy, spinocerebellar atixia, Huntington's disease, etc.) [4–7], as well as some forms of cancer [8–11]. Though these are intriguing data, we know very little about the etiology of the increased frequency of diseases in the offspring of older fathers. Among the most likely contributing factors to this phenomenon are epigenetic alterations in the sperm that can be passed on to the offspring.

These studies are in striking contrast to the previously held dogma that the mature sperm are responsible only for the safe delivery of the paternal DNA. Intriguingly, with increased investigation has come mounting evidence that the sperm epigenome is not only well suited to facilitate mature gamete function but is also competent to contribute to events in embryonic development. It has been established that even through the dramatic nuclear protein remodeling that occurs in the developing sperm, involving the replacement of histone proteins with protamines, some nucleosomes are retained [12]. Importantly, histones are retained at promoters of important genomic loci for development, suggesting that the sperm epigenome is poised to play a role in embryogenesis [12]. In addition, recent reports suggest that hypomethylated regions with high CpG density also appear to drive nucleosome retention [13]. Similarly, DNA methylation marks in the sperm have been identified that likely contribute to embryonic development as well [12,14]. These data strongly support the hypothesis that the sperm epigenome is not only well suited to facilitate mature sperm function, but that it also contributes to events beyond fertilization.

Looking past fertilization and embryogenesis, sperm appear to contribute to events manifesting later in life. The remarkable claim that sperm, independent of gene mutation, may be capable of affecting phenotype in the offspring was initially proposed as a result of large retrospective epidemiological studies observing

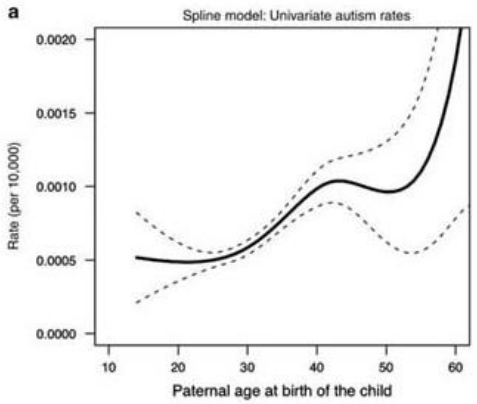
RISING AGE OF FATHERS AND INCREASED INCIDENCE OF NEUROPSYCHIATRIC DISORDERS

Schizophrenia



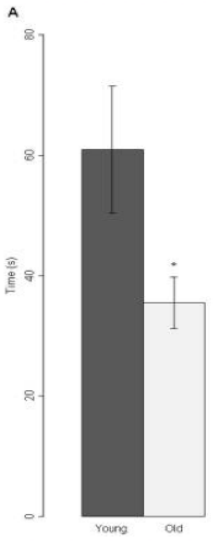
Miller et al., 2011

Autism



Gardener et al., 2009

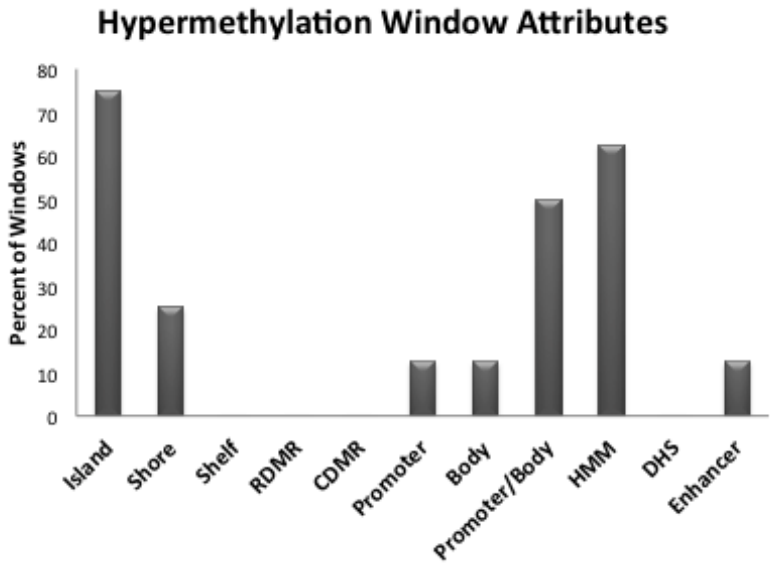
Social Behaviors

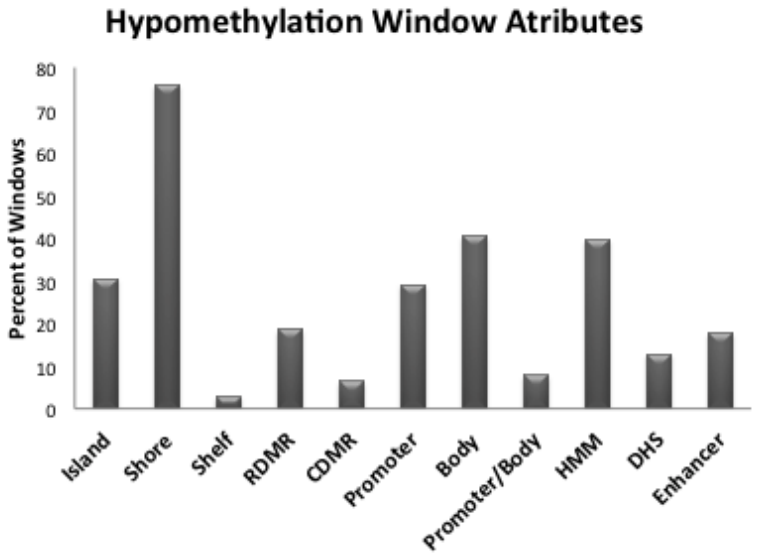


Smith et al., 2009

LOCI AFFECTED BY ADVANCING MALE AGE

- Hypermethylation
  - 8 windows
- Hypomethylation
- 139 windows

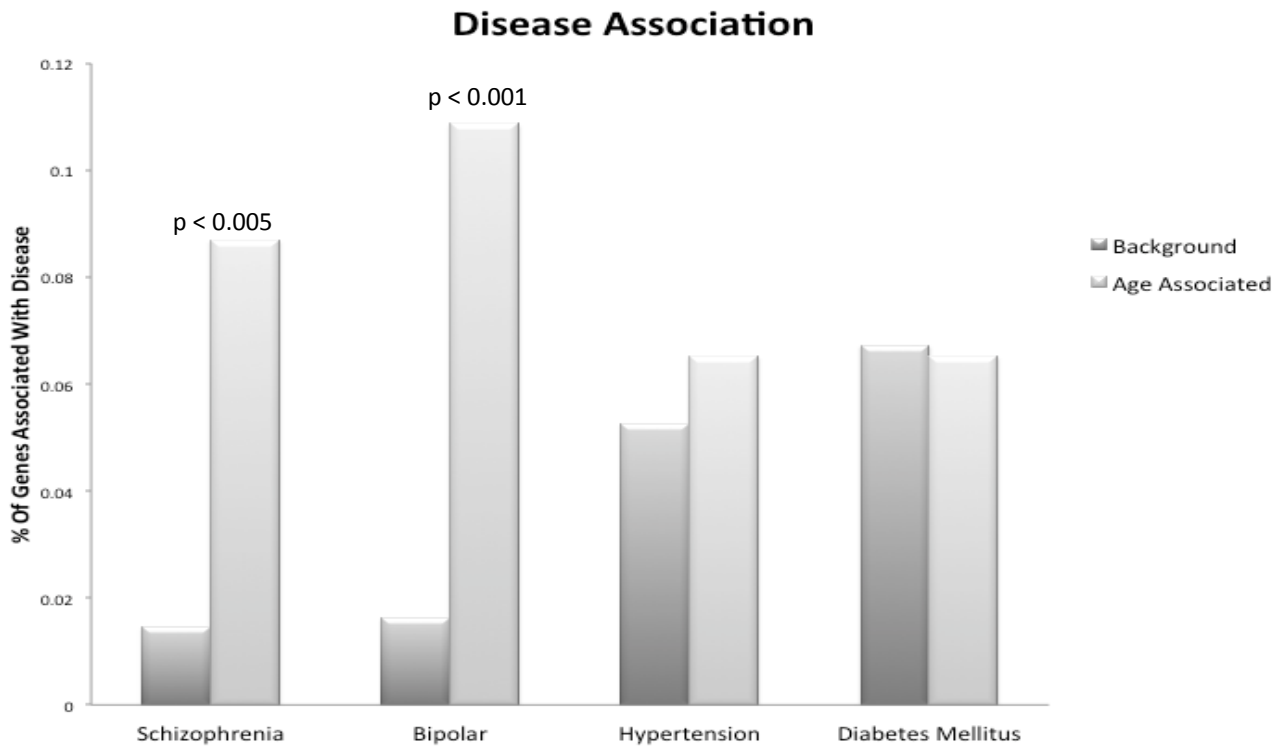




Jenkins et al., 2014

GENES/DISEASES ASSOCIATED WITH ALTERED METHYLATION DURING MALE AGING

- All diseases that are associated with at least 3 of the genes altered with age were included in our frequency analysis



Jenkins et al., 2014

CONFIRMATION OF FINDINGS

- Targeted sequencing confirmed findings

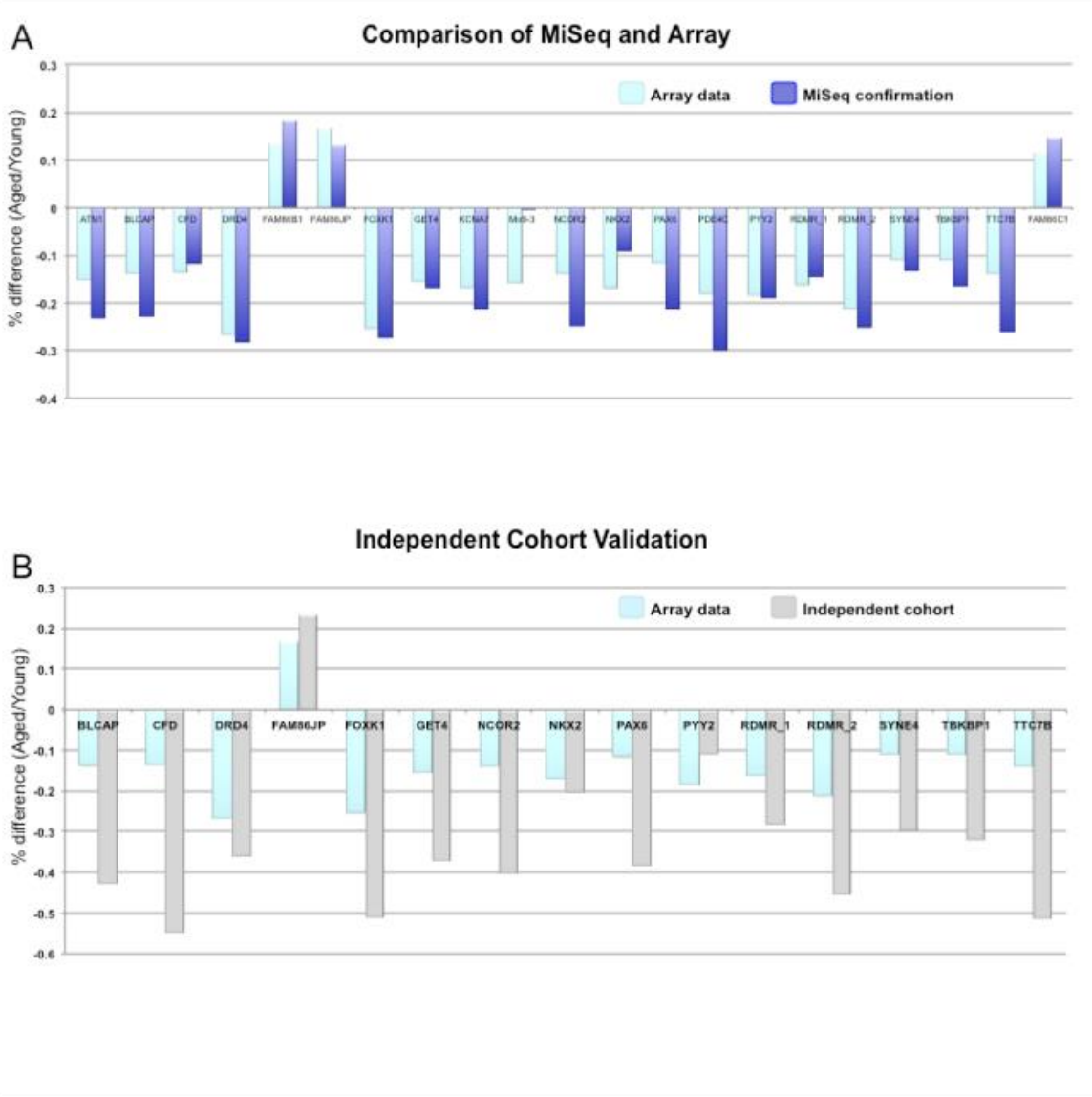
  Sequencing and array agree

- Independent cohort confirmed paired data

  <25 (n=47) vs. >45 (n=19)

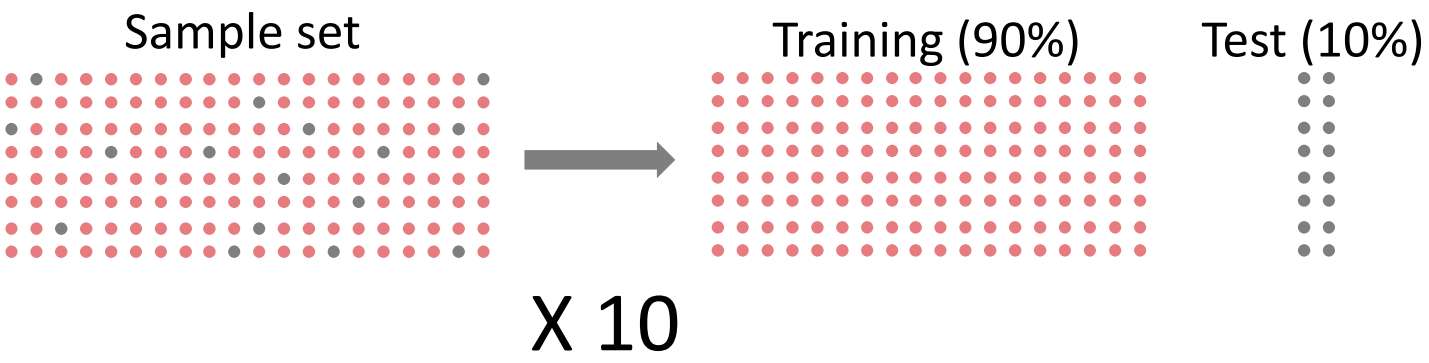
- Magnitude of change supports conclusions

  -Δ between age is **2.3 times greater** in independent cohort than in fertile controls.



AGING CALCULATOR?
BUILDING A MODEL

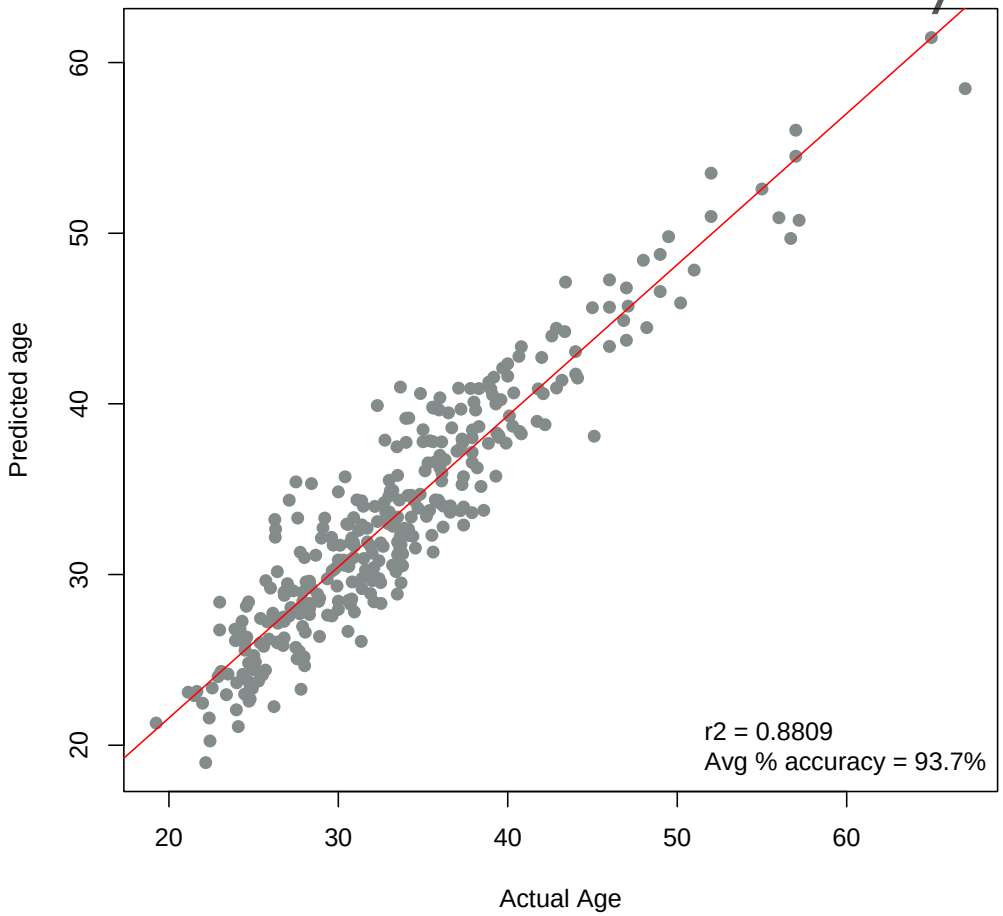
- Technical details:
  - Training a predictive model with 147 regions of interest on a dataset with 329 samples from 450k array data:
  - Utilizing a linear regression machine learning platform
    - R application – glmnet
    - Lasso and Elastic Net regularization
  - 10-fold cross validation



Jenkins et al., 2018

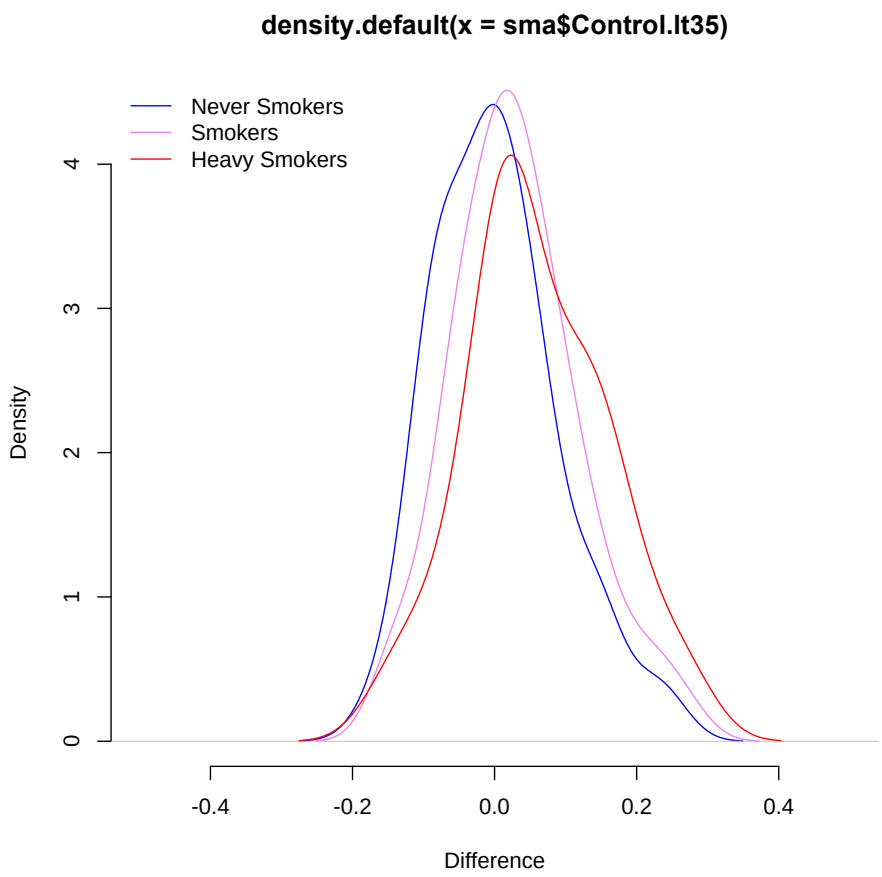
FINAL MODEL

- Includes only the heaviest weighted 51 regions and corrects for array batch.



Jenkins et al., 2018

IS THERE A POTENTIAL UTILITY?



-Could be used in the future to predict risk to offspring

A bit far off – much work still required

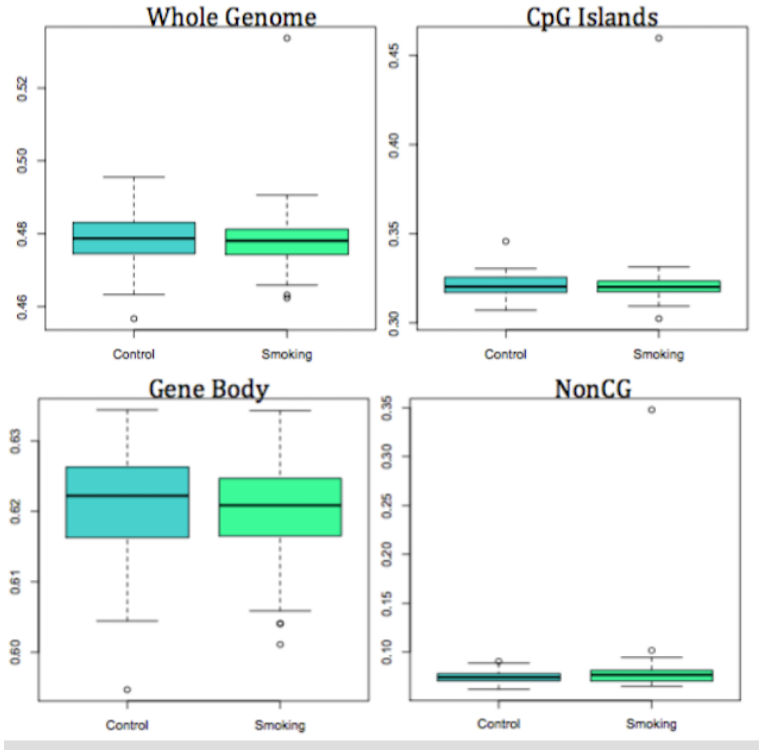
-Potential use to track interventions which may affect germ lineage in patients with accelerated aging patterns

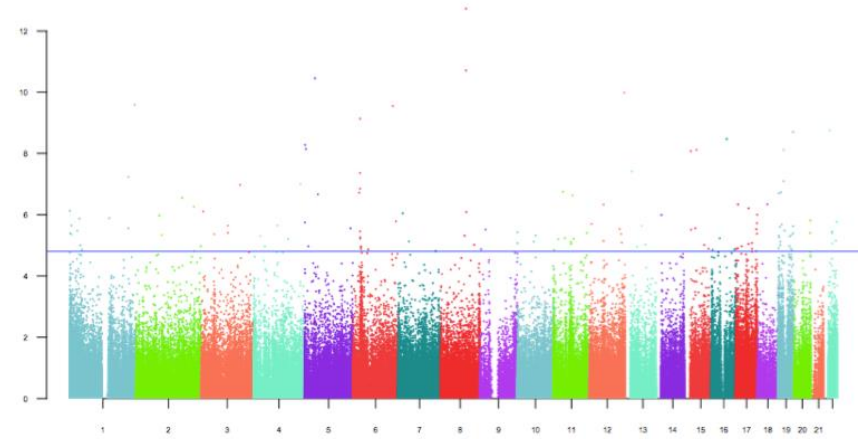
-Potentially a more powerful motivator

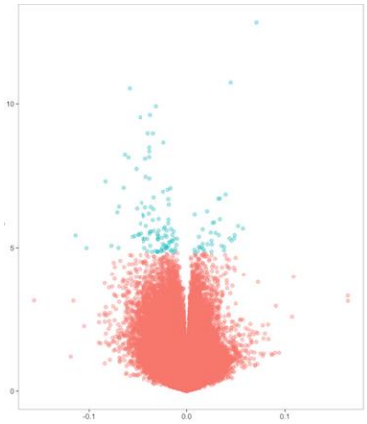
-Improved compliance?

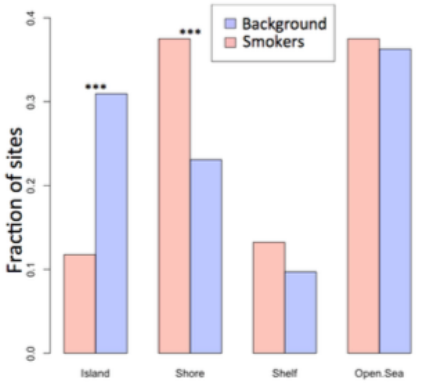
SPERM DNA METHYLATION DIFFERENCES ASSOCIATED WITH CIGARETTE SMOKING

- Methylation arrays on 78 men who smoke vs 78 never smokers

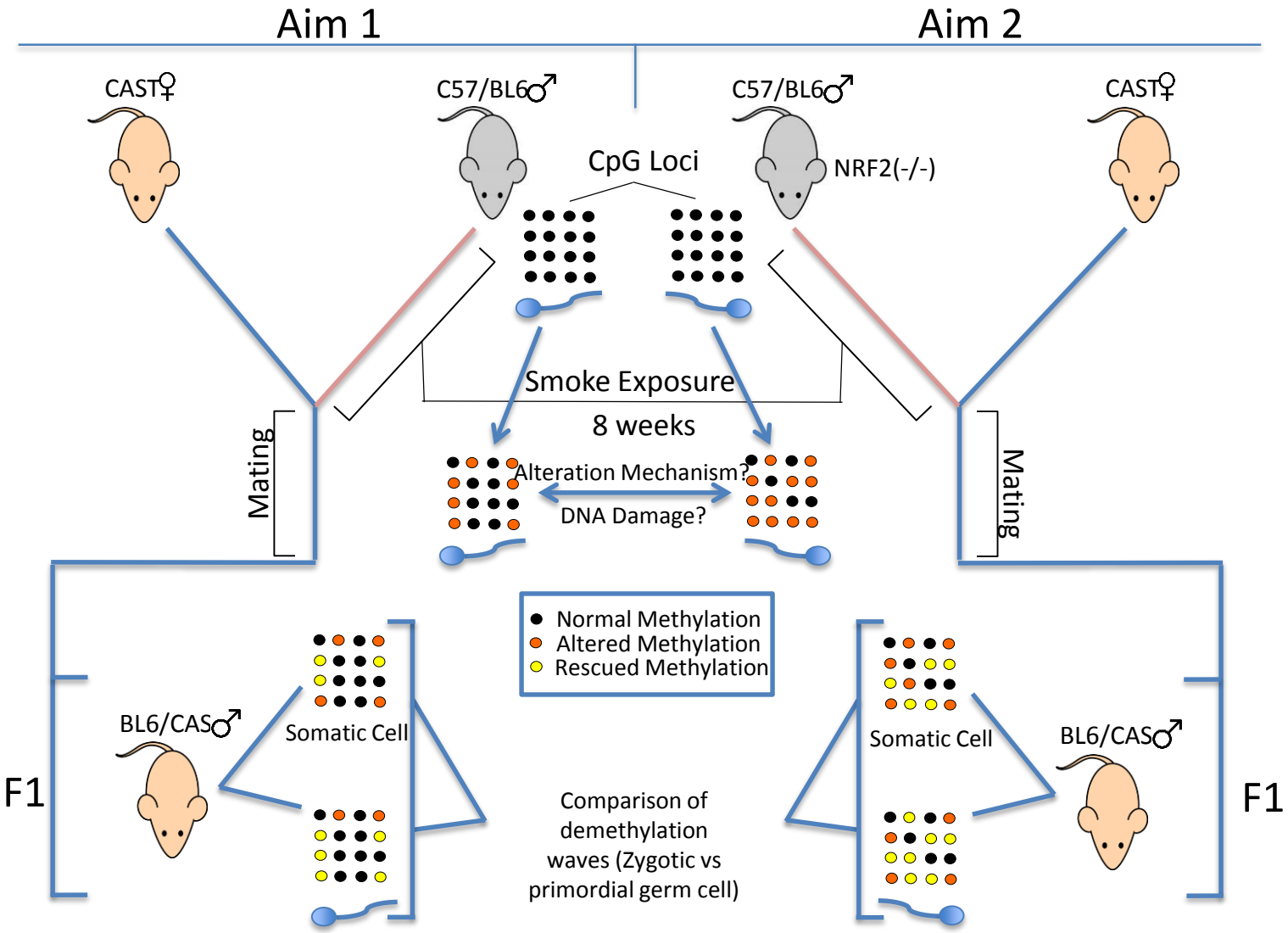








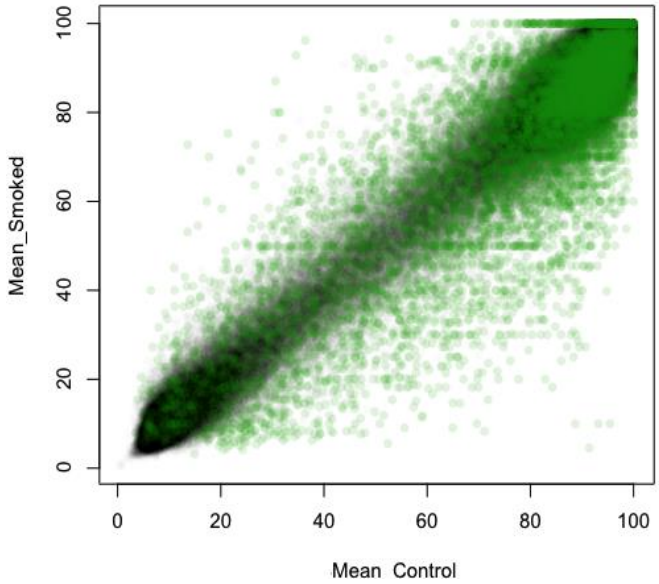
MOUSE STUDIES



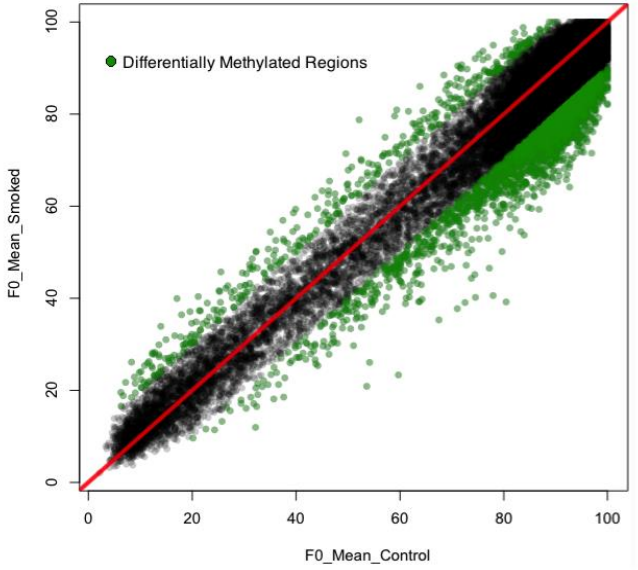
Smoking causes changes in DNAme in mouse sperm

Changes in DNAme is more dramatic in recently smoke exposed animals.

Sperm collected 3 days after smoke exposure



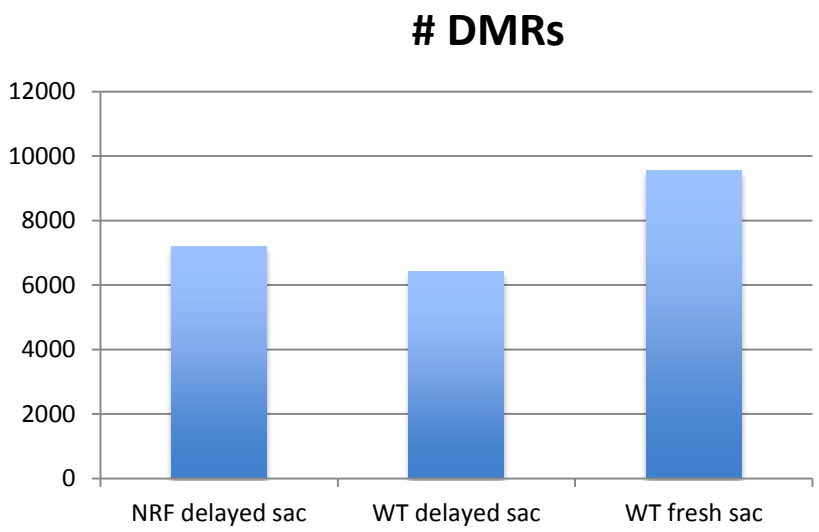
Sperm collected 50 days after smoke exposure

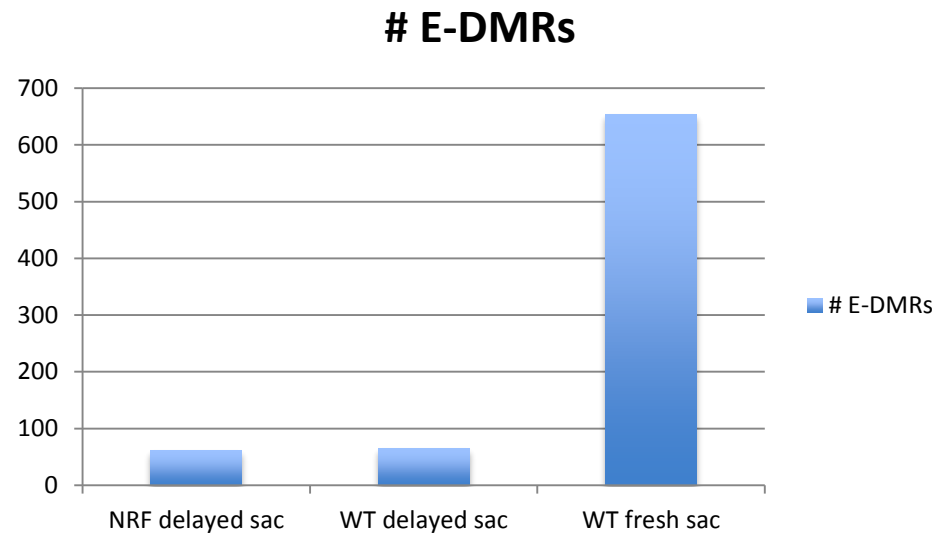


8136 decreasing and 420 increasing = 8556 DMRs (>25,000 CpGs)

DMRs = greater than 10% absolute change in DNAme and more than 3 biological replicates

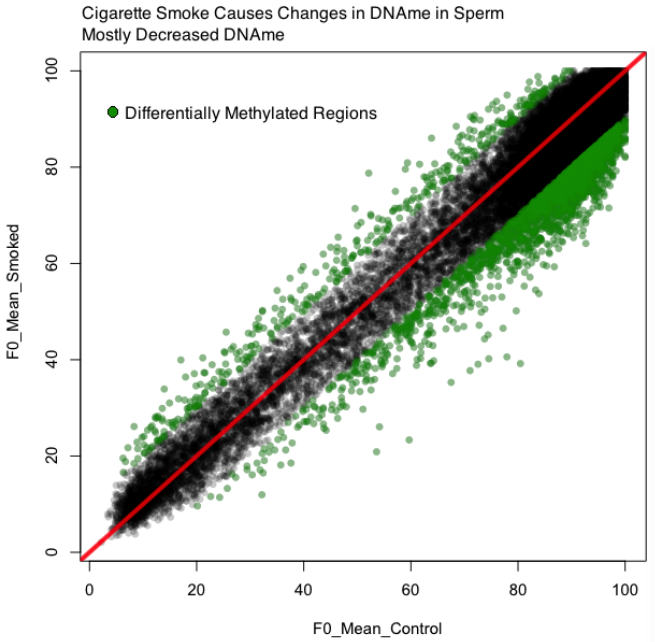
OF DMRS BY GROUP

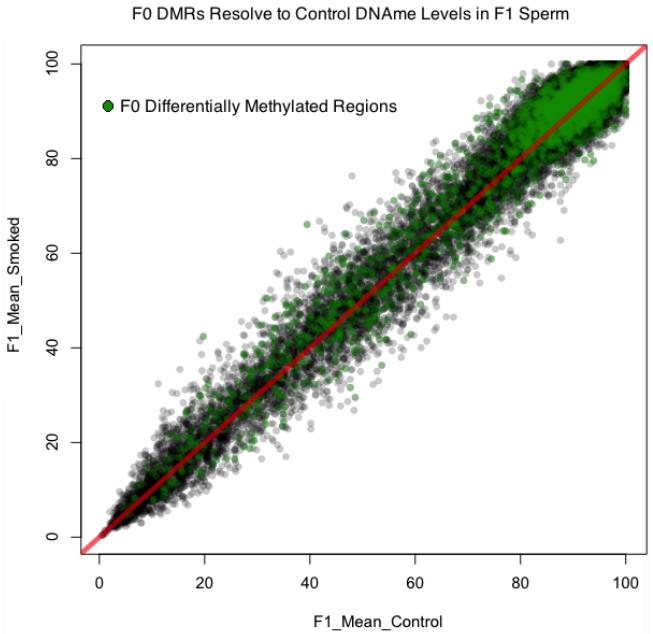




- Extreme DMRs as those with greater then 20% absolute change in DNAme

Changes in DNAme do not persist in F1 sperm samples.

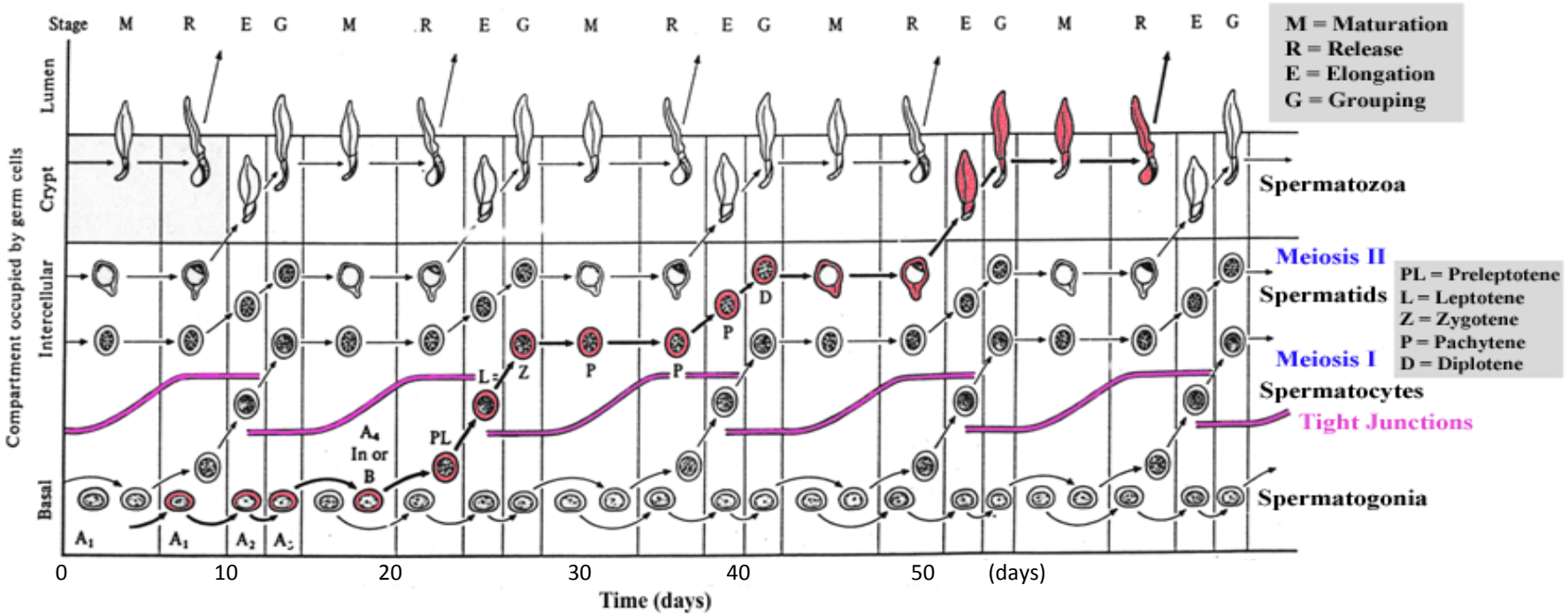




Follow up recovery experiments…

Samples collected at various times post exposure:

1. 3 days
2. 28 days (0.8 cycles)
3. 50 days (1.4 cycles)
4. 100 days (3 cycles)
5. 170 days (5 cycles)



Modified from Austin & Short, *Reproduction in Mammals, Book I: Germ Cells and Fertilization,* Cambridge University Press: Cambridge, UK, 1982.

THANK YOU

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