GENETICS AND EPIGENETICS OF MALE INFERTILITY

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

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
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.

<table>
<thead>
<tr>
<th>Congenital factors</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Anorchia</td>
<td></td>
</tr>
<tr>
<td>- Cryptorchidism</td>
<td></td>
</tr>
<tr>
<td>- Congenital Absence of Vas Deferens</td>
<td></td>
</tr>
<tr>
<td>- Genetic abnormalities (caryotype anomalies including Klinefelter syndrome; Y chromosome microdeletions; Kallmann syndrome, mutations in genes involved in Hypothalamus–pituitary–gonadal axis, Partial/Mild Androgen Insensitivity syndrome)</td>
<td></td>
</tr>
</tbody>
</table>

**Acquired factors**

- Testis trauma
- Testicular torsion
- Post-inflammatory forms (orchitis, epididymitis)
- Obstruction, subobstruction of proximal and/or distal urogenital tract
- Recurrent urogenital infections, prostatitis, prostatovesiculitis
- 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%)

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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, cKIT, BMP4, BMP8

Sperm Function
- PRM1, CSNK2A2, PICH1, 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, ODC, H2BFWT
- UBE2B, OA23, DNMT3L
- RBM, BAX, BRDT
- ACE, GOPC, NOS1
- LIMK2, LMTK2, USP26
- YBX2, NANO5, TSPY
- PARP2, PRDM9, SOHLH1
- CSNK2A2, DDX25, JMJ1A
- H1FNT, TAF7L, TEX15
- TBPL1, TNP2, GRTH
- FASLG, FAS, CFTR
- MTRR, YSSK4, PYGO2
- IL1B, MTHFR, HSF2
- TSSK2,4,6, UTP14, UBR2
- TNF, BCL2L2, TASSR38
- HMGB2, PPP1CC, OR2W3
- STYX, PACRG, KLK2
- XPC, PGAM4, ET5, PMS2
SNP GENOME-WIDE ASSOCIATION STUDIES

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

Zhibin Hu1, Yanling Xie2, Xiaoxia Cao1, Juncheng Dai1, Honggang Li1, Hongyu Hu2, Yue Song1, Feng Lu4, Tianshu Wu3, Xiaoyang Yang3, Haojie Li1, Bing Yao3, Chunsheng Lu2, Chencheng Xiong1, Zheun Li1, Yaoting Cai5, Jiayin Liu2, Zuomin Zhou2, Hongqing Shen1, Xinran Wang1, & Jiabao Shal1,2

Nature Genetics. Volume 49, Number 2 | 2017

Association studies article
Low frequency germline variants across 6p22.2–6p23.3 are associated with non-obstructive azoospermia in Han Chinese men

Bixian Ni1,2, Yuxin Lin3,4, Liangdan Sun1,2, Meng Zhu1,2, Zheng Li4, Hui Wang5, Jun Yu6, Xuejia Gao7,8, Xianbo Zuo1,2, Jing Dong1, Yankei Xie1,3, Yang Wen1,3, Hao Wu1,3, Honggang Li1, Hongyu Hu2, Ping Ping1, Xiangfeng Chen1, Juncheng Dai1, Yue Jiang1,2, Peng Xu1, Qian Du1, Bing Yao1, Ning Weng1, Hui Lu4, Zhuyang Wang6, Xiaojian Zhu5, Xiaoyu Yang1, Chenliang Xiong1, Hongxia Ma4, Guangfu Jin1, Jianfeng Xu1, Xinran Wang1, Zuomin Zhou2, Jiayin Liu1,2, Xuejia Gao7,8, Donald F. Conrad1,2, Zhibin Hu1,2,3 & Jiabao Shal1,2,4

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

Chuncheng Lu1,3, Maofei Xu4,5, Rong Wang6, Yufeng Qin6,7, Jing Ran8, Wei Wu9, Ling Song9, Shouli Wang10, Zuomin Zhou2, Hongqing Shen1, Jiabao Shal1, Zhibin Hu6, & Yankei Xie1

Mitochondrion. Volume 24, September 2015, Pages 87–92
Copy number variation associated with meiotic arrest in idiopathic male infertility

Stefanie Egger, Ph.D., Katho Hein, D.D., Jocelyn van den Berg, B.Sc., Levinia Gordon, M.Sc., Stefan J. White, Ph.D., Duangpoon Jamai, Ph.D., Robert L. McInerney, Ph.D., Andrew H. Sinclair, Ph.D., and Dietrich Konietzko, M.D.

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

Anne Fröhling, Ph.D., Peter H. Vogt, Ph.D., Jutta Zierz, Martina Welsh-Baumgartner, Ph.D., Christine Schuth, M.D., Johanna Schücker, Ph.D., M.D., Gerhard Michael Frings, M.D., and Dieter Konietzko, M.D.

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

Carlos J. Jorgez, Ph.D., 1, 2 Nathan Wilken, B.S., 1, 2 Josephine B. Addie, B.S., 1, 2 Justin Newkirk, Ph.D., 1, 2 Ilma V. Vangpaisand, M.S., 1, 2 Alexander W. Padyukov, M.D., Ph.D., 1, 2 Samiruddha Mukherjee, Ph.D., 1, 2 Jeff A. Rosenfield, M.S., 1, 2 Larry L. Lipshultz, M.D., 1, 2 and Dolores J. Lamb, Ph.D., 1

Copy number variations in testicular maturation arrest

A. Haider, P. Kumar, M. Jain, V. K. Iyer
COMPLEXITY OF SPERMATOGENESIS
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 \textit{in vitro} and \textit{in vivo} validation
INCREASING ACCESSIBILITY TO LARGE-SCALE SEQUENCING:

Cost per Genome

Moore's Law

National Human Genome Research Institute

geno.gov/sequencingcosts
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
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

Identifying variants that are damaging to gene function

CADD (Kircher, et al. 2014 Nature Genetics)
SIGNIFICANCE TESTING

Disease population

Non-disease population
THE MOTIVATING CASE:
30 YR OLD NOA MAN WITH UPD2

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)

"enormous potential of reverse genetics to expand the field of functional human genetics"

Dawn of the Human Knockout Project

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

<table>
<thead>
<tr>
<th>Center</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRT</td>
<td>296</td>
<td>78</td>
</tr>
<tr>
<td>AUS</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>DEN</td>
<td>91</td>
<td>0</td>
</tr>
<tr>
<td>WashU</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>422</td>
<td>84</td>
</tr>
</tbody>
</table>
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?
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

Aberrant sperm DNA methylation predicts male fertility status and embryo quality


**Objective:** The role of sperm DNA methylation in the prediction of male fertility status and embryo quality is an important area of research. This study aimed to investigate the relationship between sperm DNA methylation patterns and male fertility status.

**Methods:** Sperm samples were obtained from healthy men with no known fertility issues. Sperm DNA methylation patterns were analyzed using next-generation sequencing technology.

**Results:** The study found that sperm DNA methylation patterns were significantly associated with male fertility status. Specifically, aberrant methylation patterns were observed in sperm samples from men with fertility issues, compared to those with normal fertility status.

**Conclusion:** The findings suggest that sperm DNA methylation patterns may serve as a biomarker for predicting male fertility status and embryo quality. Further research is needed to validate these findings and to understand the underlying mechanisms.

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Decreased fecundity and sperm DNA methylation patterns


**Objective:** The role of sperm DNA methylation in the prediction of male fertility status and embryo quality is an important area of research. This study aimed to investigate the relationship between sperm DNA methylation patterns and male fertility status.

**Methods:** Sperm samples were obtained from healthy men with no known fertility issues. Sperm DNA methylation patterns were analyzed using next-generation sequencing technology.

**Results:** The study found that sperm DNA methylation patterns were significantly associated with male fertility status. Specifically, aberrant methylation patterns were observed in sperm samples from men with fertility issues, compared to those with normal fertility status.

**Conclusion:** The findings suggest that sperm DNA methylation patterns may serve as a biomarker for predicting male fertility status and embryo quality. Further research is needed to validate these findings and to understand the underlying mechanisms.
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 | © The Author 2017. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com
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 | © The Author 2017. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com
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## SUPPORTING STUDIES

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Loci</th>
<th>Sperm Count</th>
<th>Morph. / Motil.</th>
<th>Fertility</th>
<th>Pregnancy Outcome</th>
<th>DNA fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benchaib et al., Hum. Reprod. 2004</td>
<td></td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Yes</td>
<td>---</td>
</tr>
<tr>
<td>Houshdaran et al., PLoS ONE, 2007</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Urdinguio et al., Hum. Reprod. 2015</td>
<td></td>
<td>--</td>
<td>--</td>
<td>Yes</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Montjean et al., Andrology, 2015</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>--</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Loci</th>
<th>Semen params.</th>
<th>Fertility</th>
<th>Embryo dev. / preg. / miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marques et al., Mol. Hum. Reprod., 2008</td>
<td>H19, MEST, IGF2</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Wu et al., PLoS ONE, 2010</td>
<td>MTHFR</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Hammoud et al., Fertil. Steril., 2010</td>
<td>LIT1, MEST, SNRPN, PLAGL1, PEG3, H19, and IGF2</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>El Hajj et al., Sex Dev., 2011</td>
<td>H19, GTL2, LIT1, MEST, NESPAS, PEG3, SNRPN, ALU, LINE1</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Ankolkar et al., Fertil. Steril., 2012</td>
<td>H19</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Xu et al., Andrologia, 2016</td>
<td>MEST, GNAS, H19, FAM508, LINE-1, P16</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Poplinski 2010 (Int. J. Andro.)</td>
<td>IGF2/H19 ICR1, MEST</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Urdinguio et al., Hum. Reprod. 2015</td>
<td>ALU repeats, 2752 CpGs (~1800 genes; ~60 imprinted)</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Kuhz et al., Epigenetics, 2014</td>
<td>GTL2</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Xu et al., Biol. Reprod., 2013</td>
<td>Pebp1</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>
## Conclusions:

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

1 IN 10 COUPLES ARE INFERTILE,

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

50% of IVF treatment cycles fail, even when using IVF-ICSI

22% of infertility is of unknown cause (unexplained infertility)

EMBRYO VIABILITY / QUALITY
GOOD
MODERATE
POOR

40
Summary

**Semen Analysis**
(based on concentration threshold of 13.5 X 10^6 / ml, the best performing parameter threshold in this study.)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>14.8%</strong></td>
<td>%</td>
<td><strong>96.1%</strong></td>
</tr>
<tr>
<td><strong>SENSITIVITY</strong></td>
<td>Percentage</td>
<td>% Percentage of known fertile men identified as fertile</td>
</tr>
<tr>
<td><strong>SPECIFICITY</strong></td>
<td>% Percentage of suspected infertile men classified as infertile</td>
<td></td>
</tr>
</tbody>
</table>

**DNA Methylation Profile for Fertility**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>84.3%</strong></td>
<td></td>
<td><strong>92.1%</strong></td>
</tr>
</tbody>
</table>

**DNA Methylation Profile for embryo quality**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>50.0%</strong></td>
<td></td>
<td><strong>94.0%</strong></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Org.</th>
<th>Insult</th>
<th>Sperm Epigenome impact</th>
<th>Phenotype impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donkin et al., Cell Mat., 2015</td>
<td><em>Homo sapiens</em></td>
<td>Gastric bypass-induced weight loss</td>
<td>Genes involved in regulation of appetite and weight, including FTO (also implicated in male infertility)</td>
</tr>
<tr>
<td>Denham et al., Epigenomics, 2015</td>
<td><em>Homo sapiens</em></td>
<td>Exercise intervention</td>
<td>Global changes in sperm DNA methylation; inc. genes related to schizophrenia and Parkinson’s disease</td>
</tr>
<tr>
<td>Palmer et al., Am. J. Physiol. Endocrinol. Metab., 2012</td>
<td><em>Mus musculus</em></td>
<td>Diet and exercise changes</td>
<td>Not assessed</td>
</tr>
</tbody>
</table>
RISK FACTORS:

- Biological Factors
  - Aging
  - Obesity
  - Diet
  - Cancer

- Environmental Exposure
  - Smoking
  - Alcohol
  - Cancer Therapies
  - Medications
  - Air Pollution
  - Socio-economic stress
  - Toxic waste exposure

Abstract

Recent evidence demonstrates a role for paternal aging in offspring disease susceptibility. It is well established that various neuropsychiatric disorders (schizophrenia, autism, etc.), thyroid abnormalities associated diseases (pressygyria, hypothalamic, 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 of older fathers, in most cases the mechanisms that drive these processes are unclear. However, it is commonly believed that age-related, and specifically DNA methylation alterations, play a role in this. In this study we have investigated the impact of aging on DNA methylation in rat brain samples. Using a multivariate analysis approach we have evaluated changes in DNA methylation with aging in different brain regions. Our data show that the patterns of DNA methylation in the brain are significantly affected by age. A representative subset of these alterations has been confirmed in an independent cohort. A total of 17 genes are associated with these regions of methylation alterations (expression or gene body). Interestingly, the expression of some of these genes is associated with highlighting the clinical relevance of these findings and suggesting potential therapeutic strategies. In conclusion, our data suggests that DNA methylation alterations in the brain are significantly affected by age, and these alterations may provide insight into the molecular mechanisms underlying age-related neurological disorders.
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

![Disease Association Graph](graph.png)

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**
  $\Delta$ 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

Sample set  Training (90%)  Test (10%)  

X 10

Jenkins et al., 2018
Includes only the heaviest weighted 51 regions and corrects for array batch.
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

Aim 1

CAST♀

C57/BL6♂

CpG Loci

Smoke Exposure

8 weeks

Alteration Mechanism?

DNA Damage?

Normal Methylation

Altered Methylation

Rescued Methylation

Comparison of demethylation waves (Zygotic vs primordial germ cell)

F1

Somatic Cell

BL6/CAST♂

Aim 2

C57/BL6♂

NRF2(−/−)

CAST♀

Mating

Mating
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
• Extreme DMRs as those with greater than 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)
