KING'S College LONDON

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Behavioural and Molecular Differences Associated with Advanced Paternal Age

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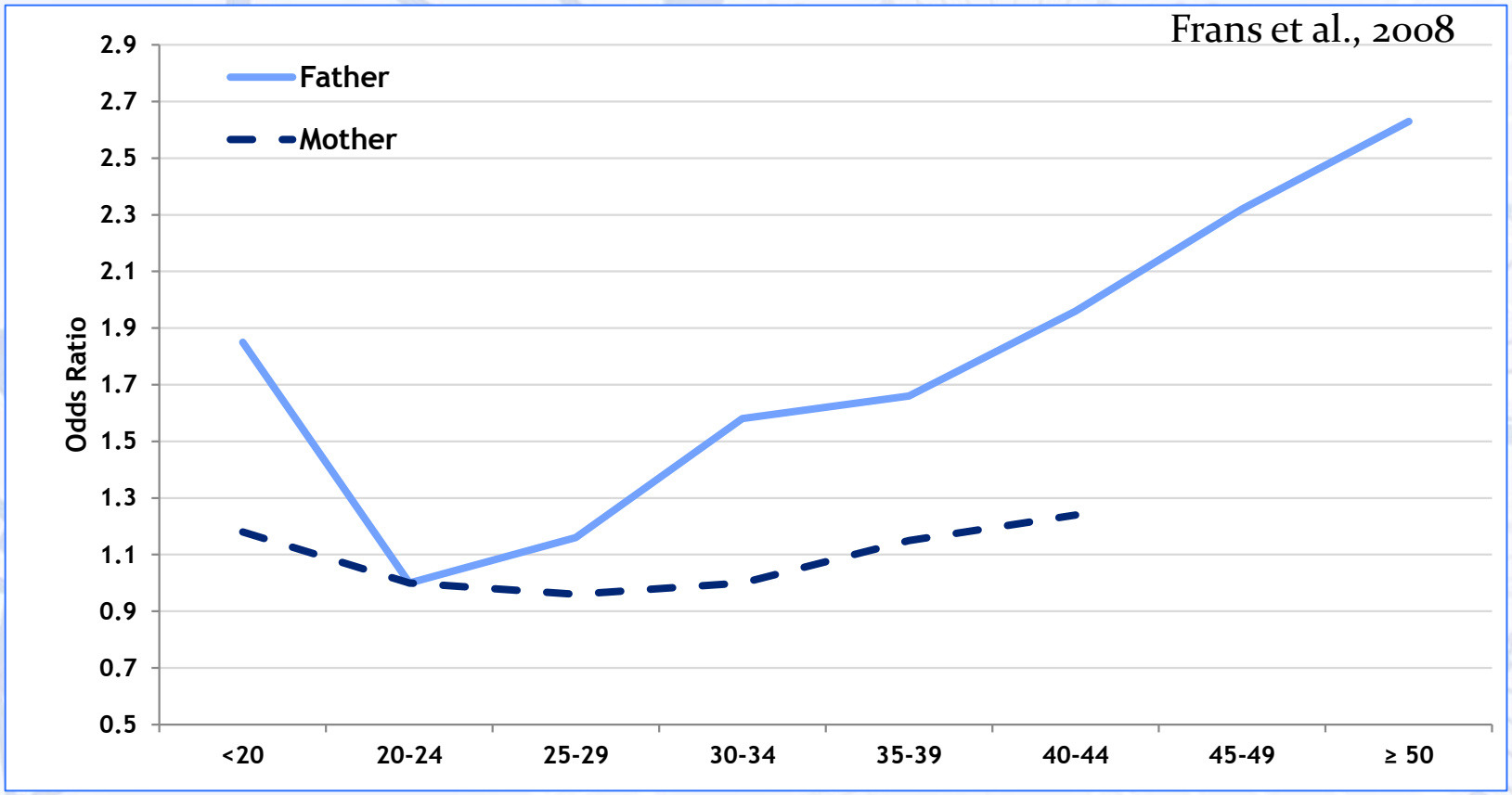
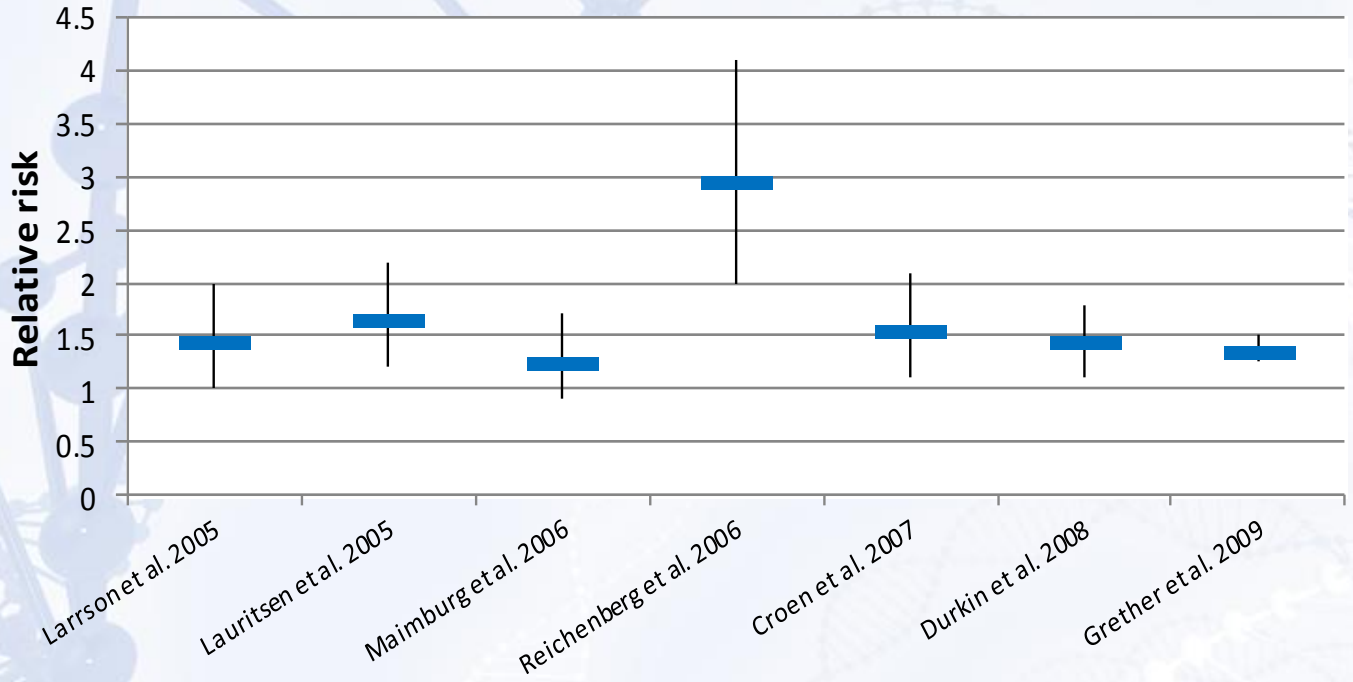
Advanced Paternal age

- Advanced paternal age is associated with autism
- Advanced paternal age also strongly linked with other conditions



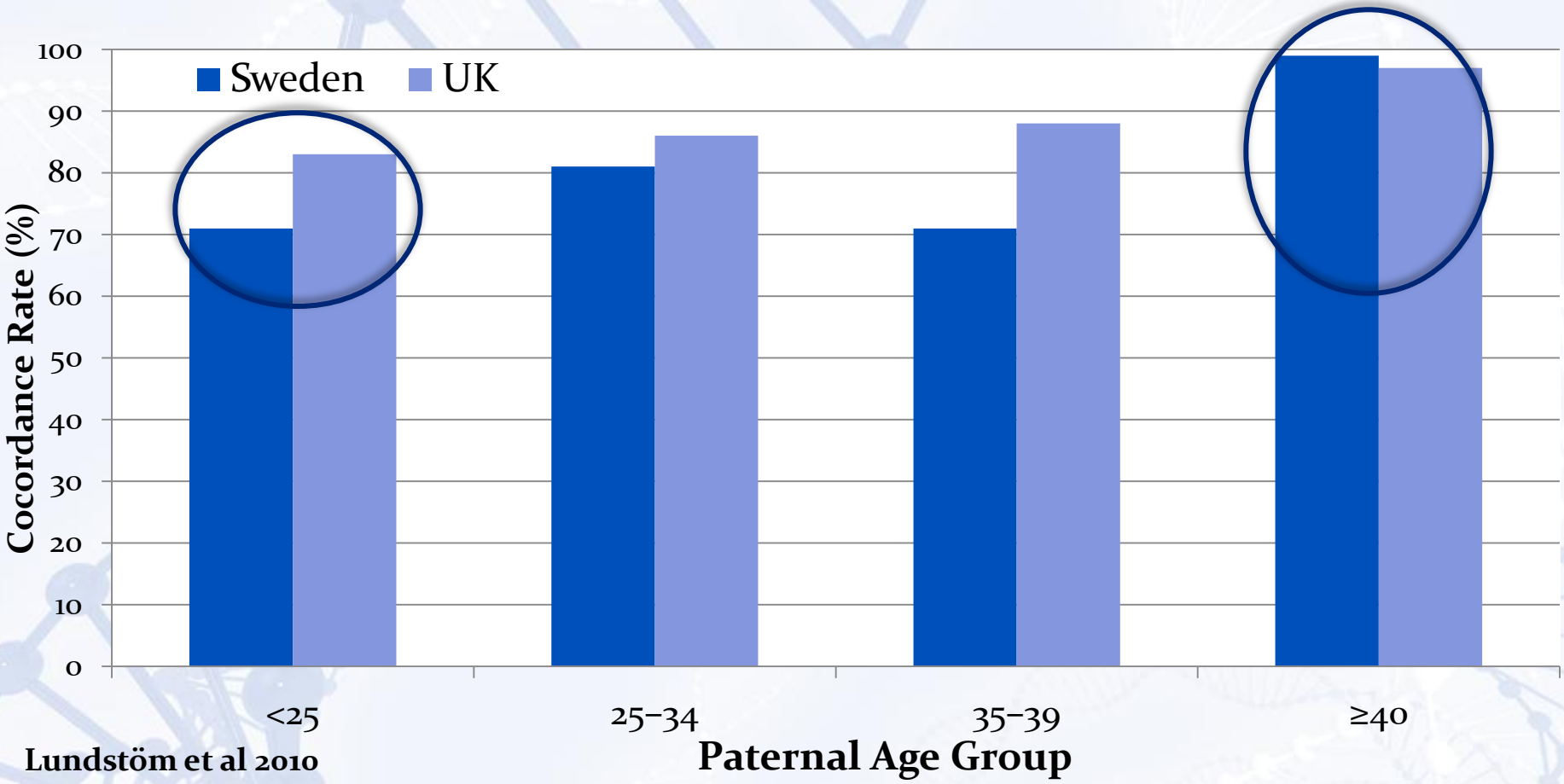
Increased Risk of Autism with Increasing Paternal Age

- Meta-analyses suggests that older age of fathers increases risk of autism by more than 50% if the fathers are 40 years or older.



- Having fathers 50 years or older more than doubles risk of developing autism (Grether et al. 2009)

Advanced Paternal Age and Autism MZ Twin Concordance

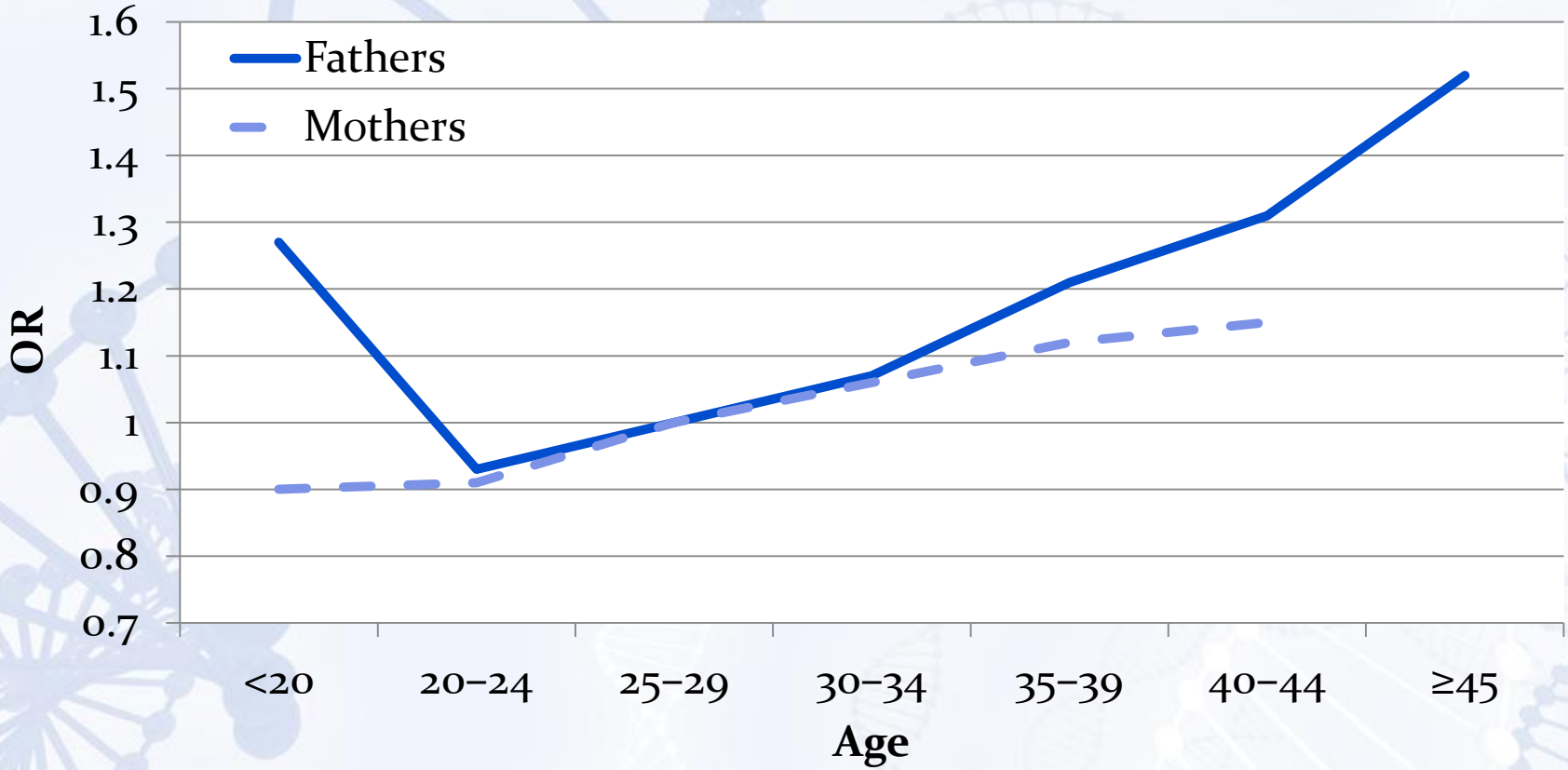


Lundstöm et al 2010

- Significant increase in twin concordance for autism with increasing paternal age

Paternal Age and Autism-Like Traits

- Advanced paternal age is associated with autism-like traits in the general population
- Strongest association is with impaired social functioning (Weiser et al., 2008; Lundstrom et al., 2010)



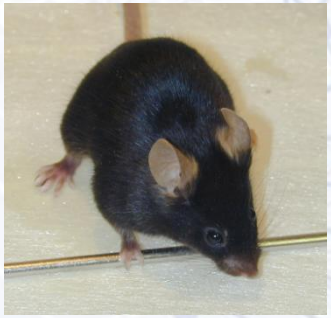
Adapted from Weiser et al., 2008

An Animal Model of Paternal Age

- Epidemiological approaches are limited to observation
- Aim of our study: to develop an animal model of advanced paternal age
  - Environmental and genetics controlled
  - Maternal age controlled
  - Elucidate underlying molecular mechanisms
- Paternal age may be related to phenotypes shared across psychiatric disorders

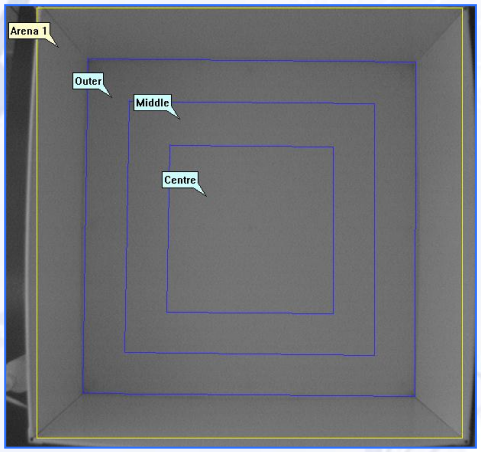
Mouse Breeding Strategy

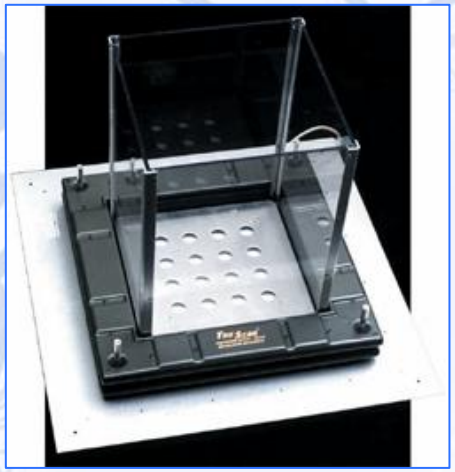
- Typical breeding age for mice starts at two months
- Male breeders are generally ‘retired’ after 7–8 months
- C57BL/6J inbred strain obtained from Charles River Laboratories
- Females aged two months were bred with males of three different ages
  - ‘Young’ (standard) males of 2 months ≈ 20 years
  - ‘Old’ males of 10 months ≈ 35-40 years
  - ‘Very Old’ males of 12 months ≈ 45-50 years
- Male offspring were housed with litter mates until 10 weeks then individually housed
- Offspring were aged 12 weeks at the start of behavioural testing



Mouse Behavioural Testing

- Open Field (locomotor activity/ exploration/anxiety)



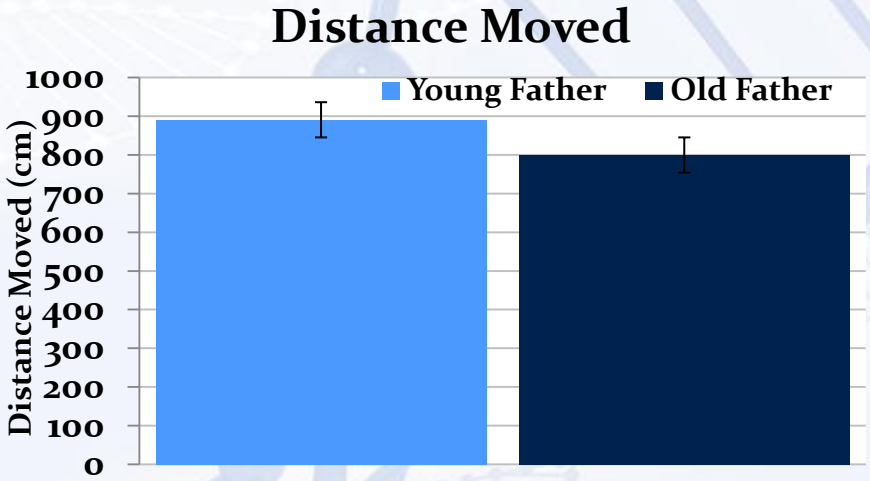


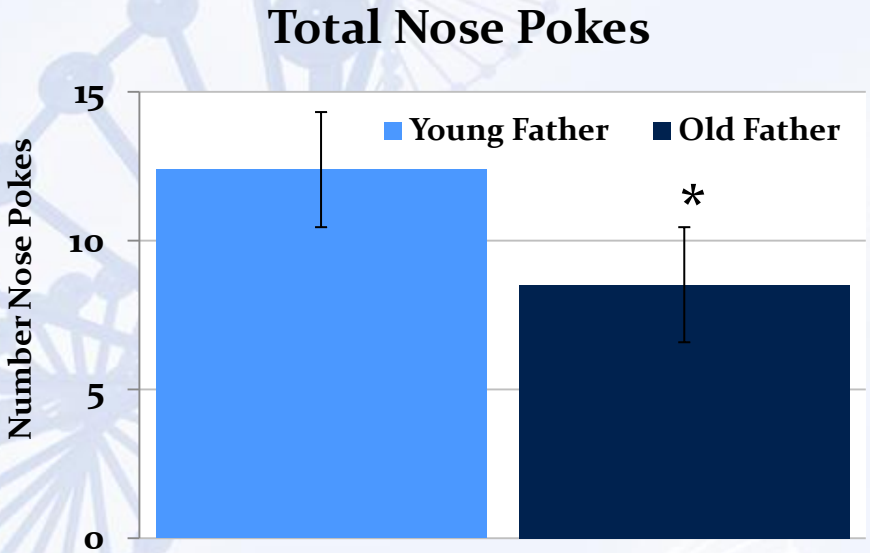
- Holeboard (exploration)

- Social behaviour (social activity)
  - Behaviour towards novel, male, juvenile C57BL/6J mouse

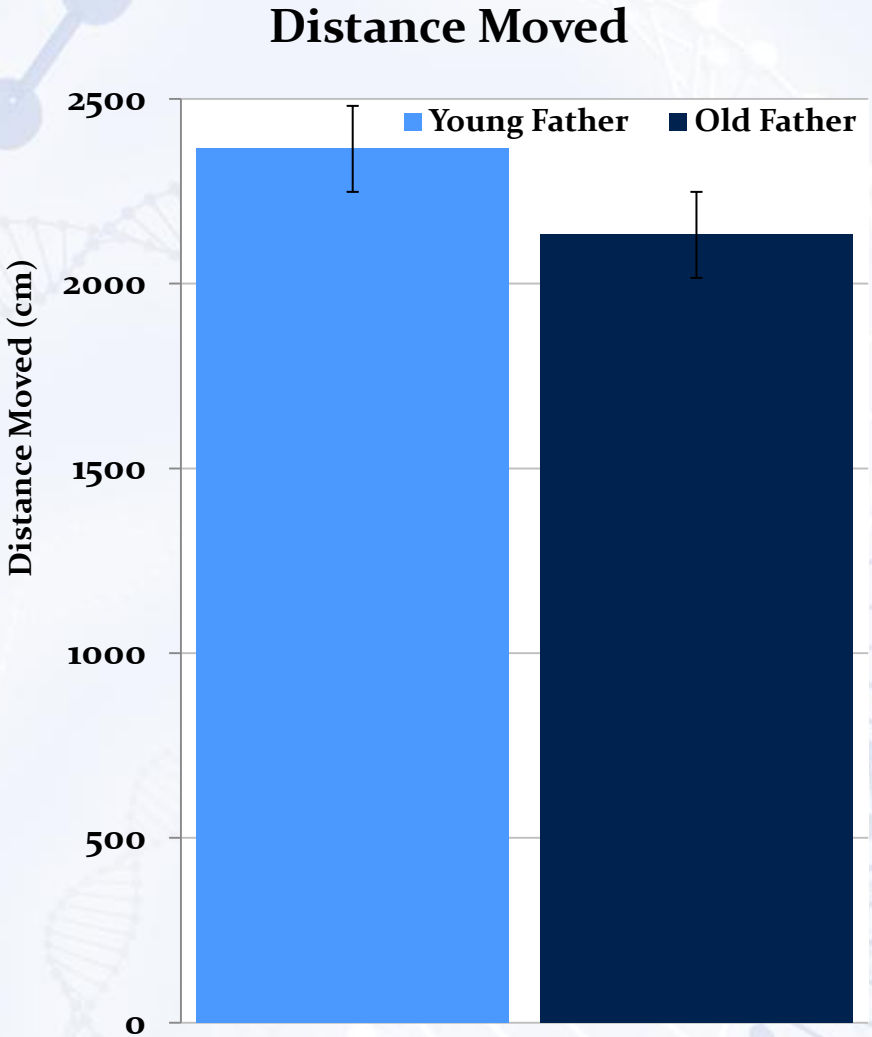
Reduced ‘Exploration’ in the Offspring of Old Fathers but No Loss of Activity

Holeboard test

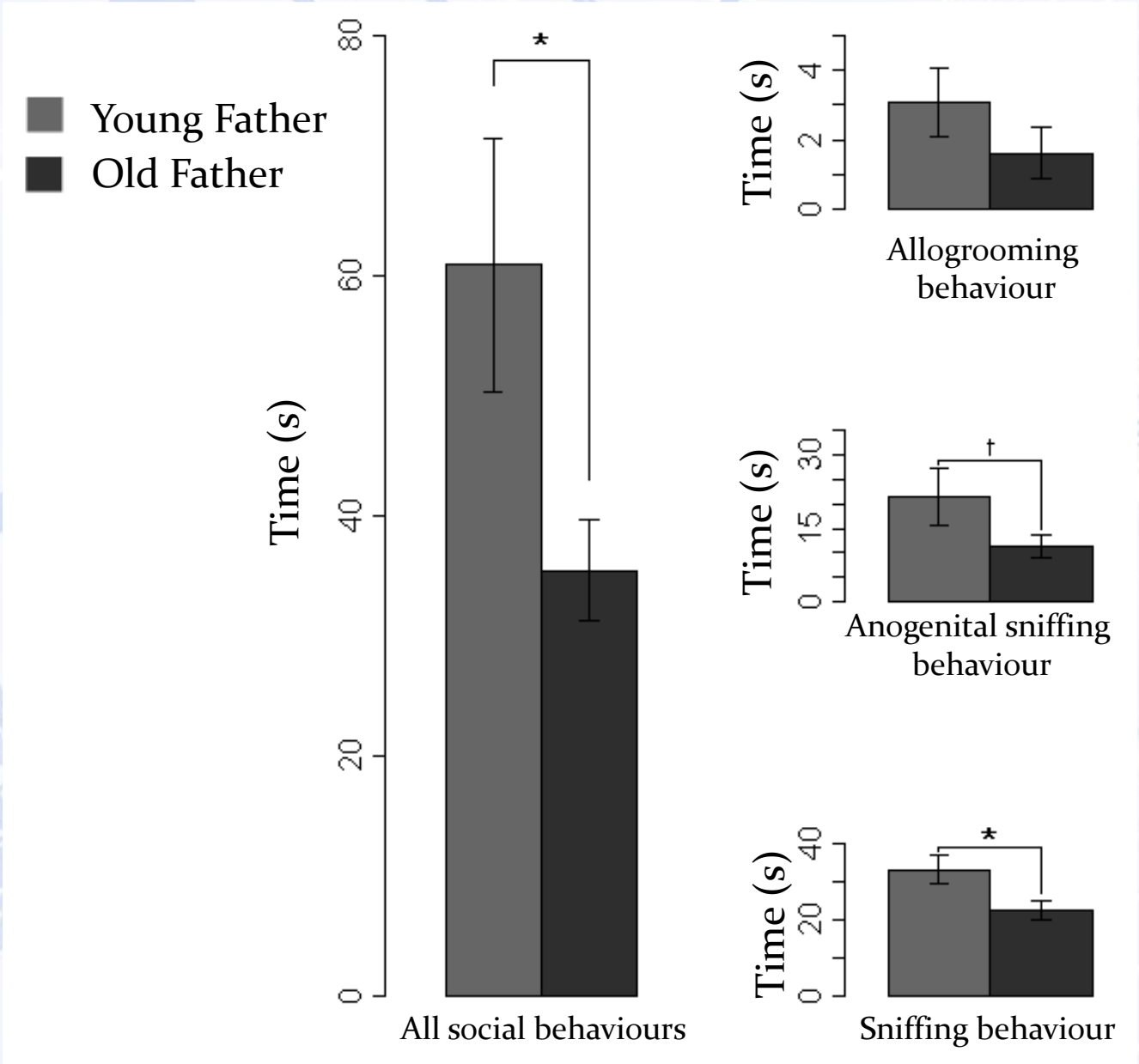




Open field



Reduced 'Social' Behaviour in the Offspring of Old Fathers

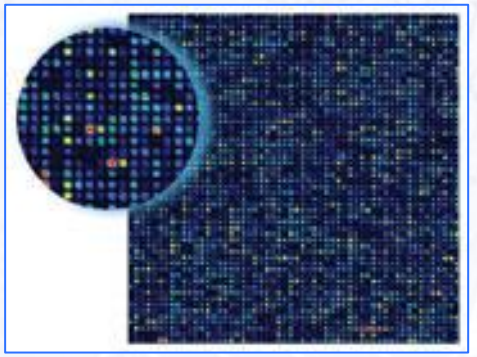


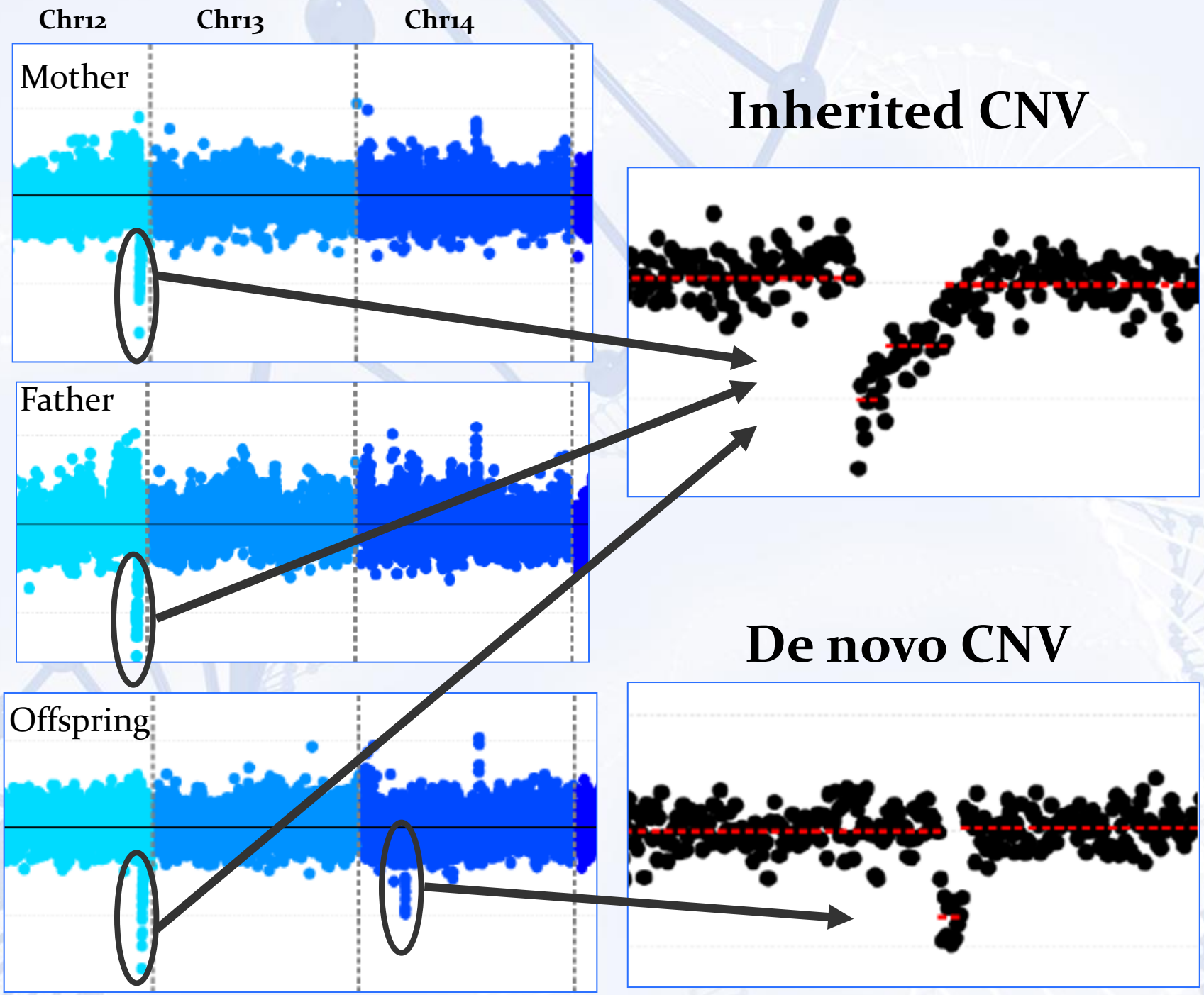
De Novo Genetic Changes?

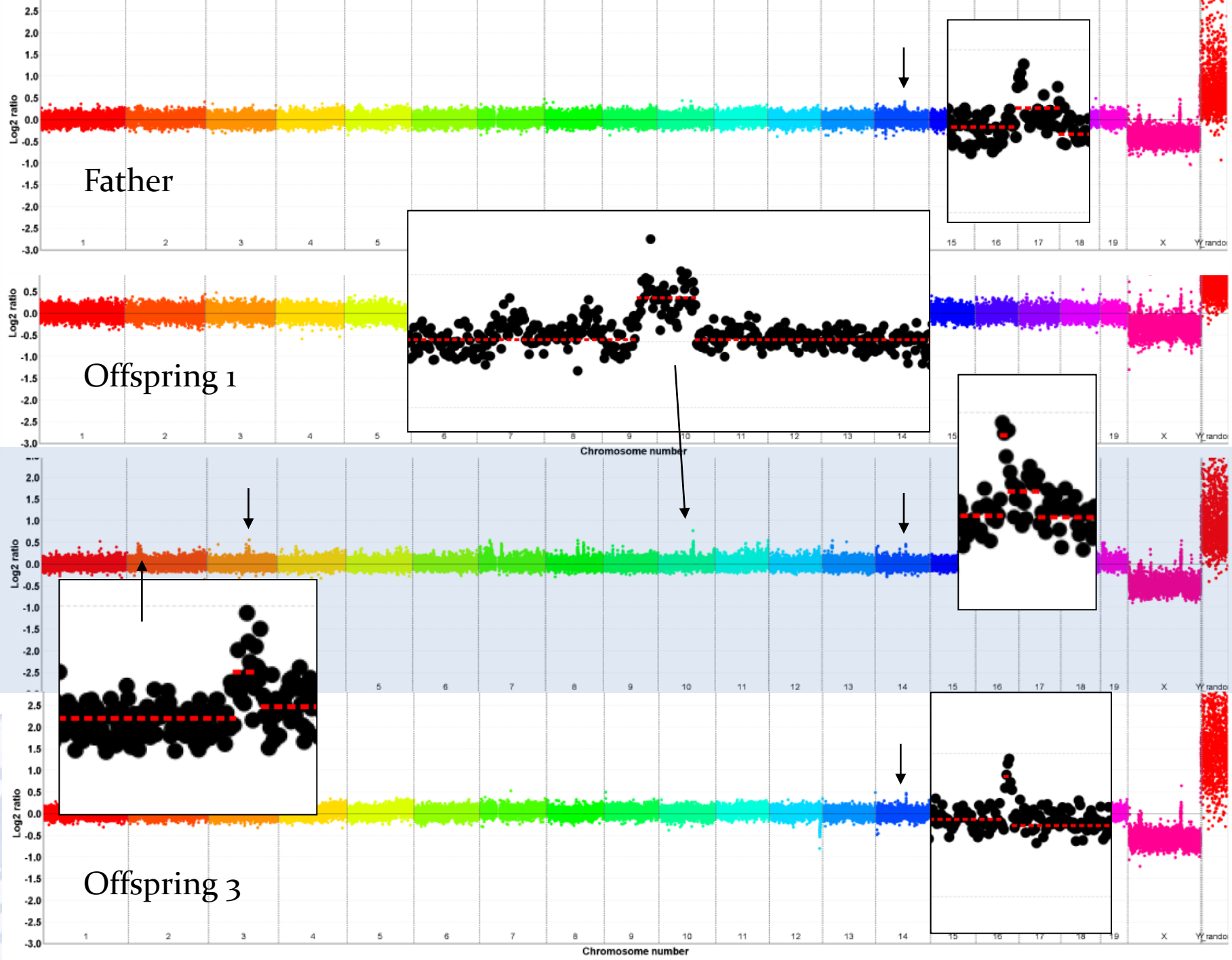
- Spermatogonial stem cell division occur over the reproductive life of men
- Hypothesised that the sperm of older men have
  - Higher rate of novel mutations (Crow, 2000)
  - More cytogenetic abnormalities (Buwe et al, 2005)
- To date, no systematic genome-wide survey in humans
- Older male mice shown to have increased germ cell mutation frequency (Walter et al, 1998)
- Interesting that numerous neurological/psychiatric phenotypes caused by genomic alteration
- Mounting evidence for a role of *de novo* CNVs psychiatric conditions

De Novo CNV Detection

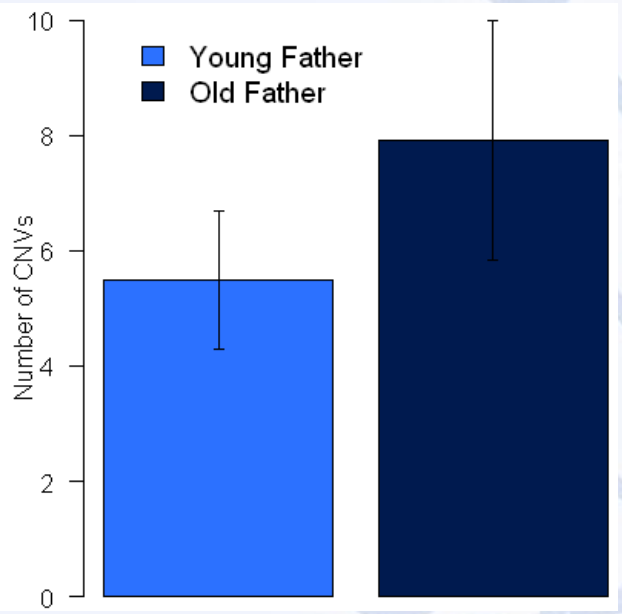
- High molecular weight DNA isolated from spleen of male breeders, female breeders and offspring
- NimbleGen CGH arrays
- Each individual hybridized against a common (female) reference individual
- Normalized log2 fluorescence intensity ratios generated using NimbleScan software using the segMNT algorithm



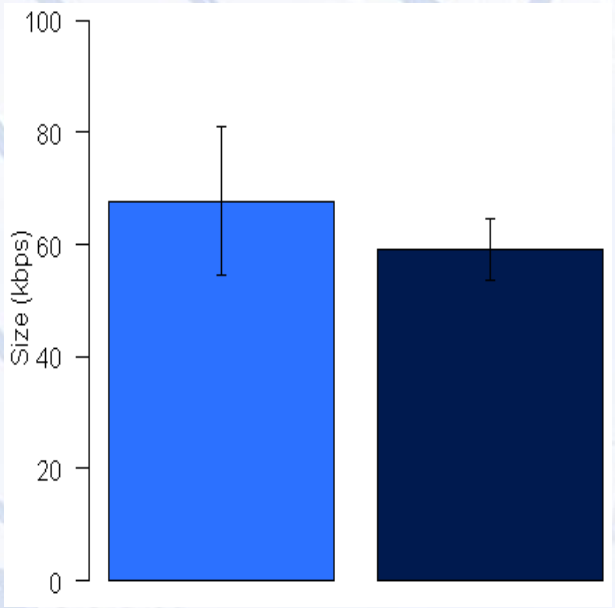

Chr12
Chr13
Chr14
Mother
Father
Offspring
Inherited CNV
De novo CNV


Father
Offspring 1
Offspring 3
Log2 ratio
Chromosome number

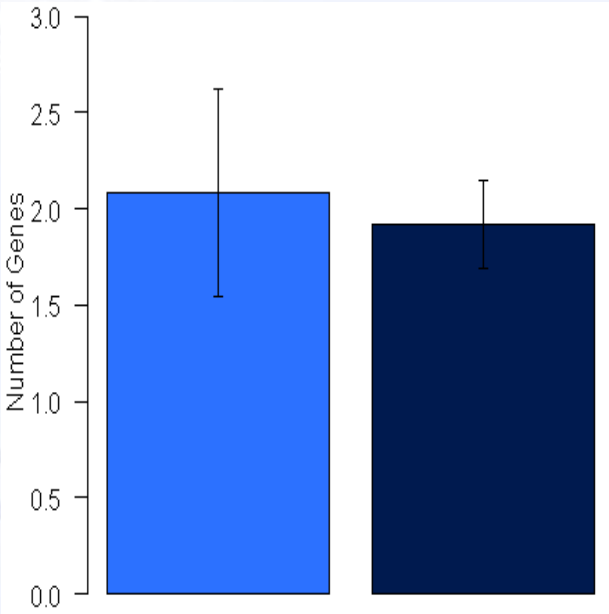
Number of CNVs



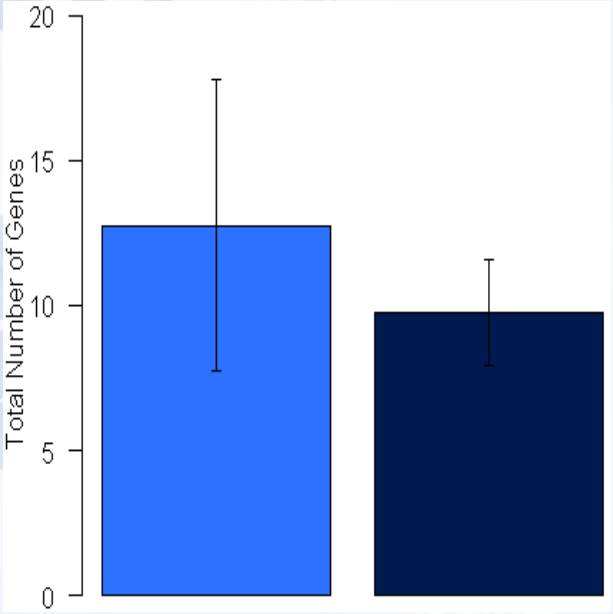
Average Size of CNVs



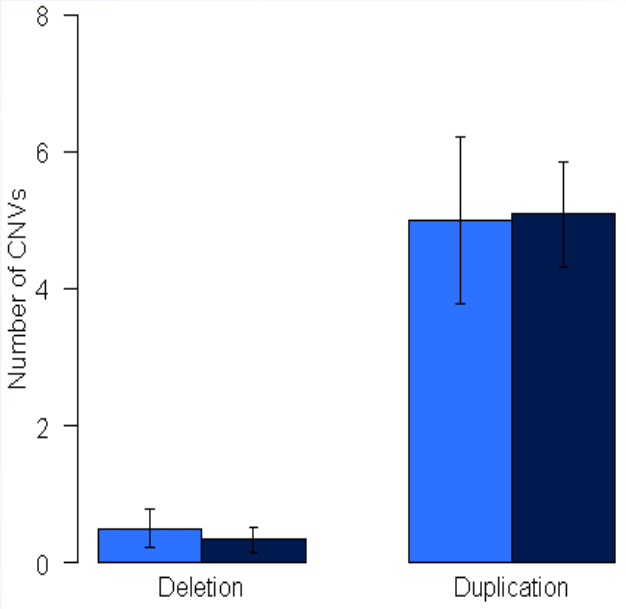
Average Genes in CNVs



Total Genes Affected by CNVs



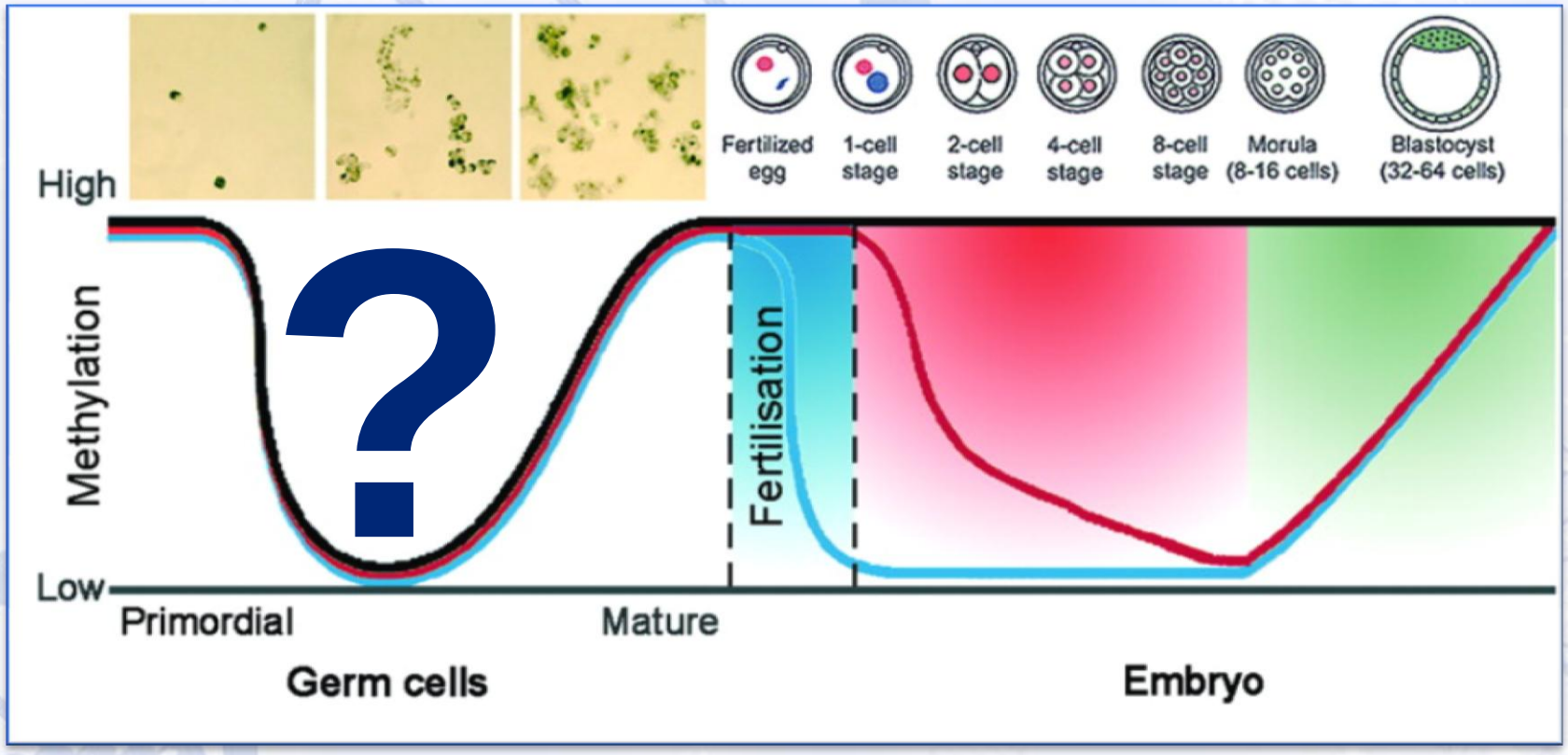
Number of Deletions and Duplications



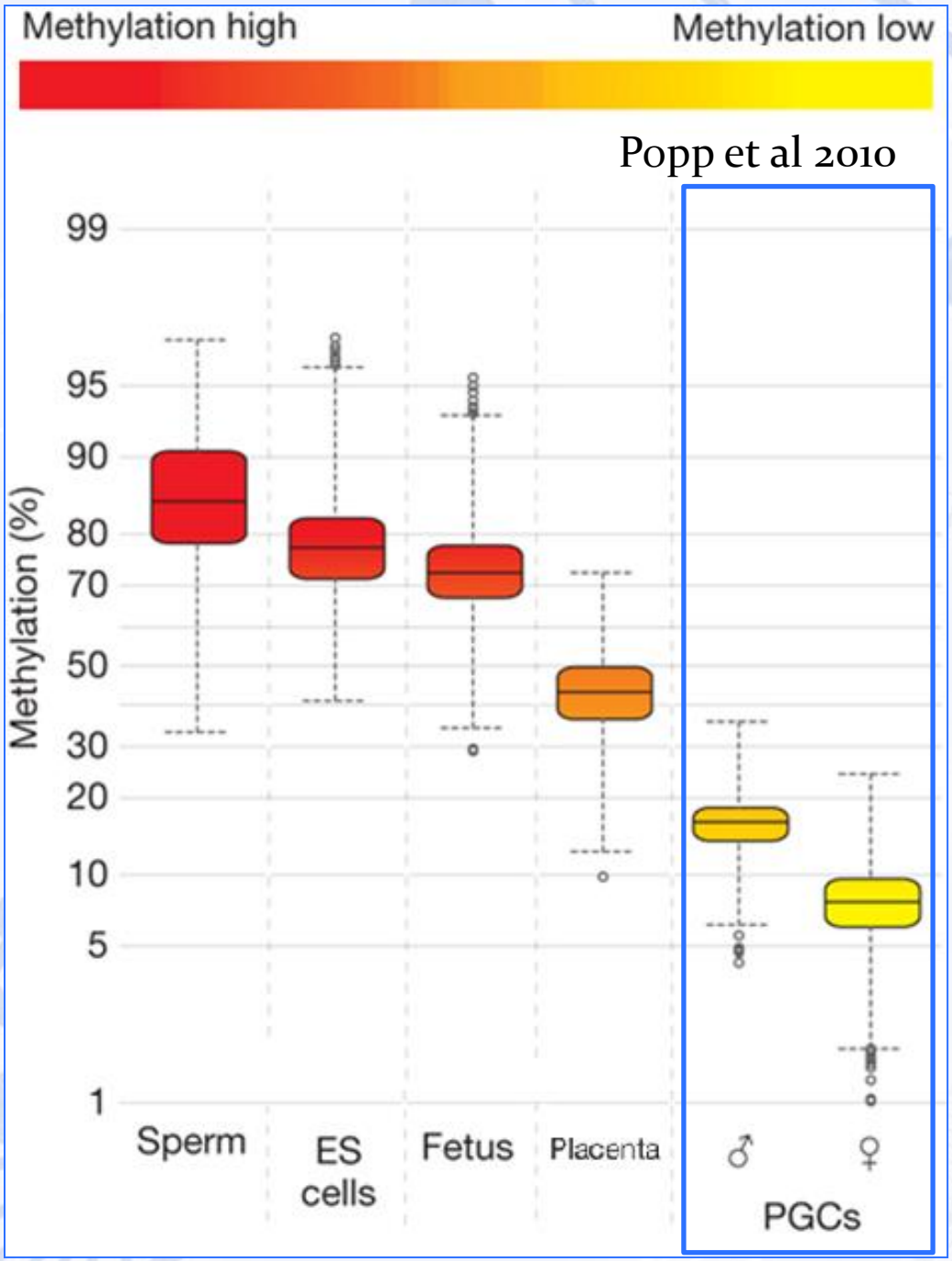
De Novo Epigenetic Changes?

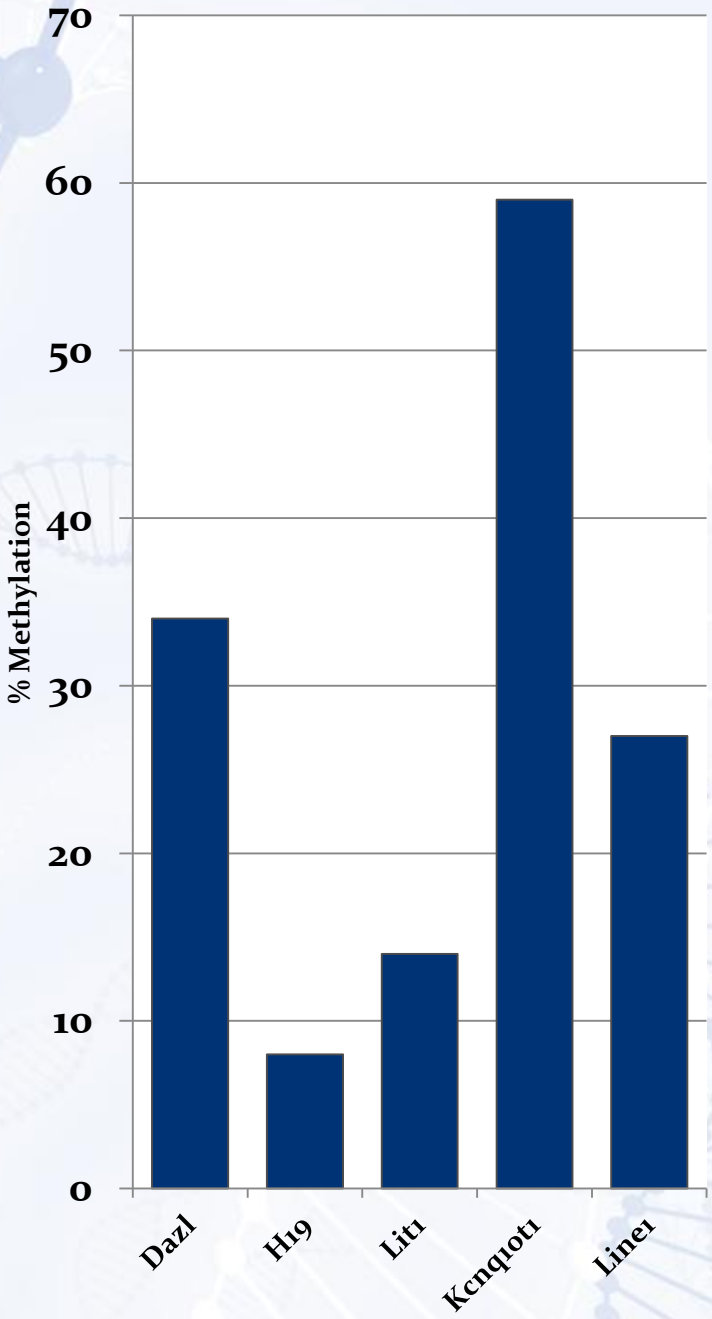
- Flanagan et al (2006): significant intra- and inter-individual epigenetic variability in the male germline
- A number of genes shown to demonstrate age-related epigenetic changes in sperm
- Stochastic and/or environmentally-induced alterations in DNA methylation propagate over cell-divisions

Epigenetic Profile Reset and Re-Established During Gametogenesis?



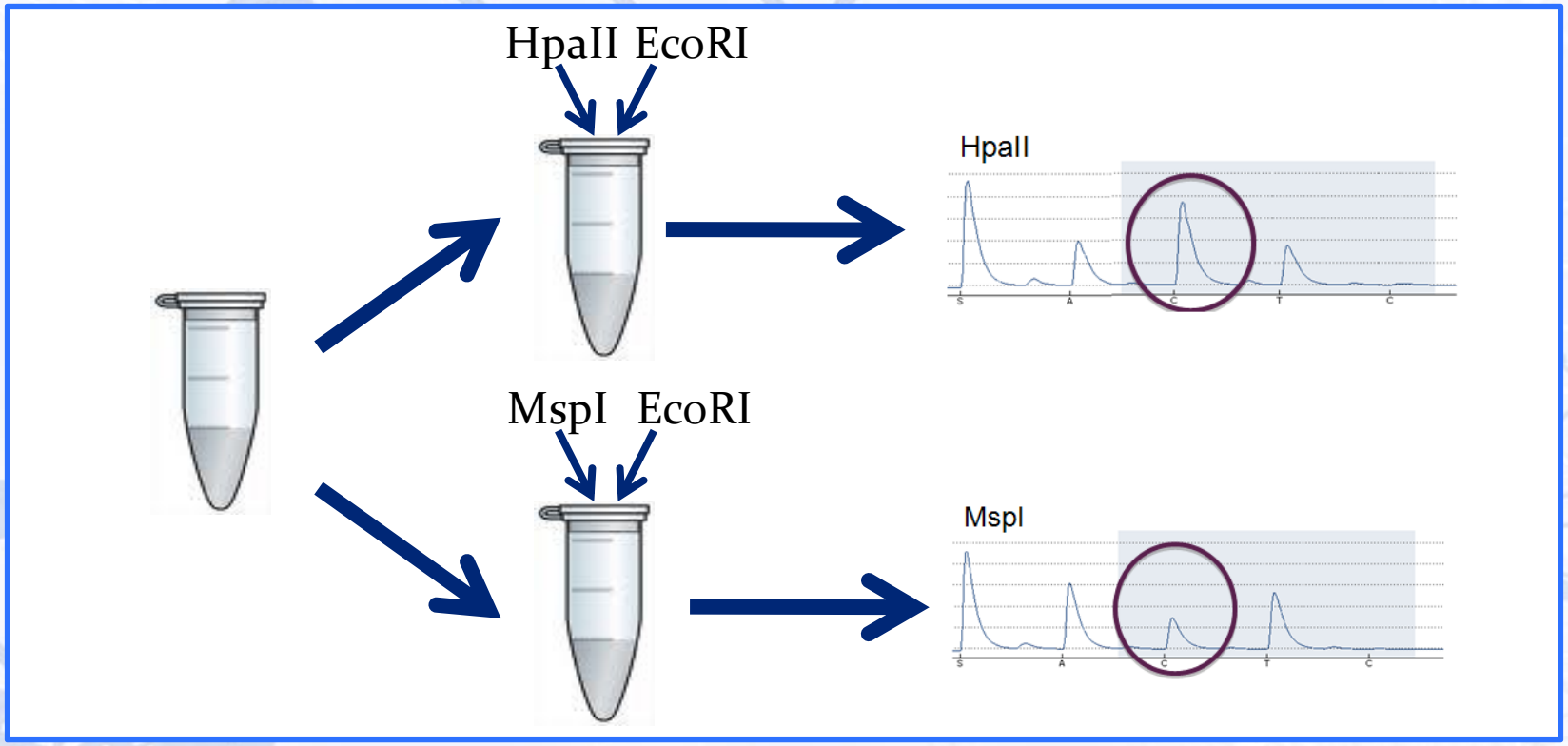
Epigenetic reprogramming not 100%


Methylation high
Methylation low
Popp et al 2010
Methylation (%)
99
95
90
80
70
50
30
20
10
5
1
Sperm
ES cells
Fetus
Placenta
♂
♀
PGCs


% Methylation
70
60
50
40
30
20
10
0
Dazl
H19
Liti
Kcnqotı
Linei

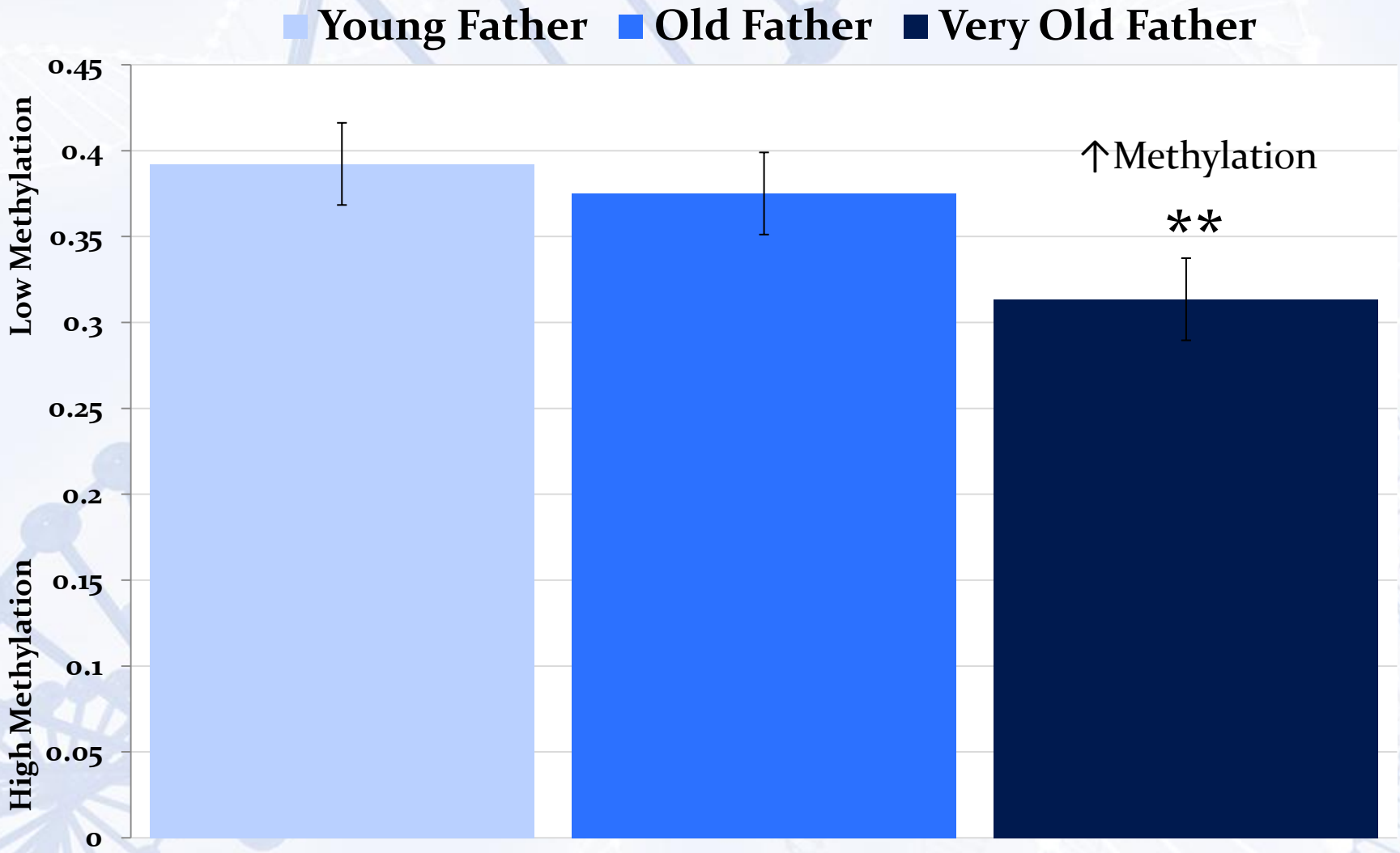
Global DNA Methylation

- Global DNA methylation was assessed using the luminometric methylation assay (LUMA (Karimi et al, 2006)

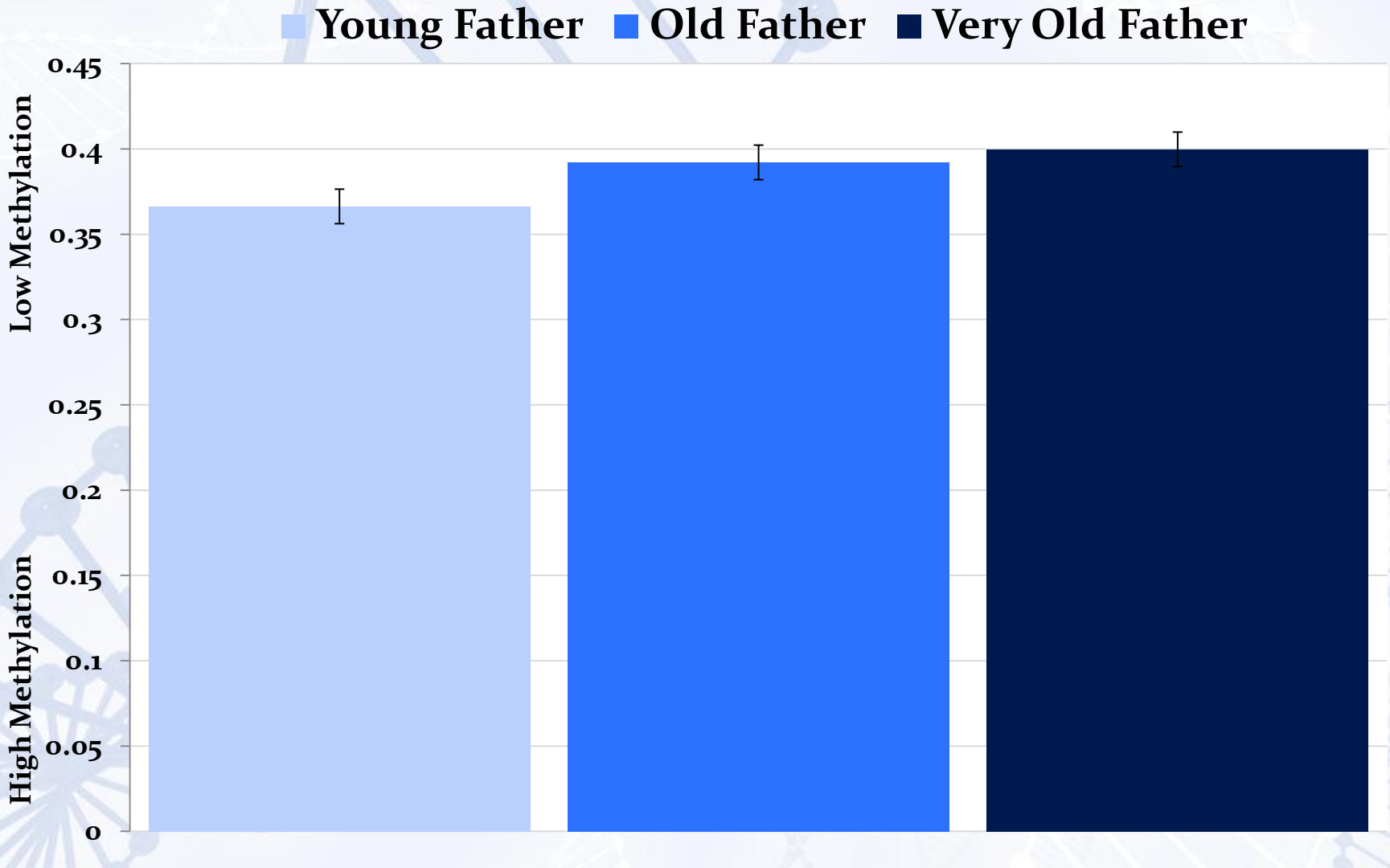


- Global methylation assessed in genomic DNA from both offspring (spleen, hippocampus and cerebellum) and parents (spleen)

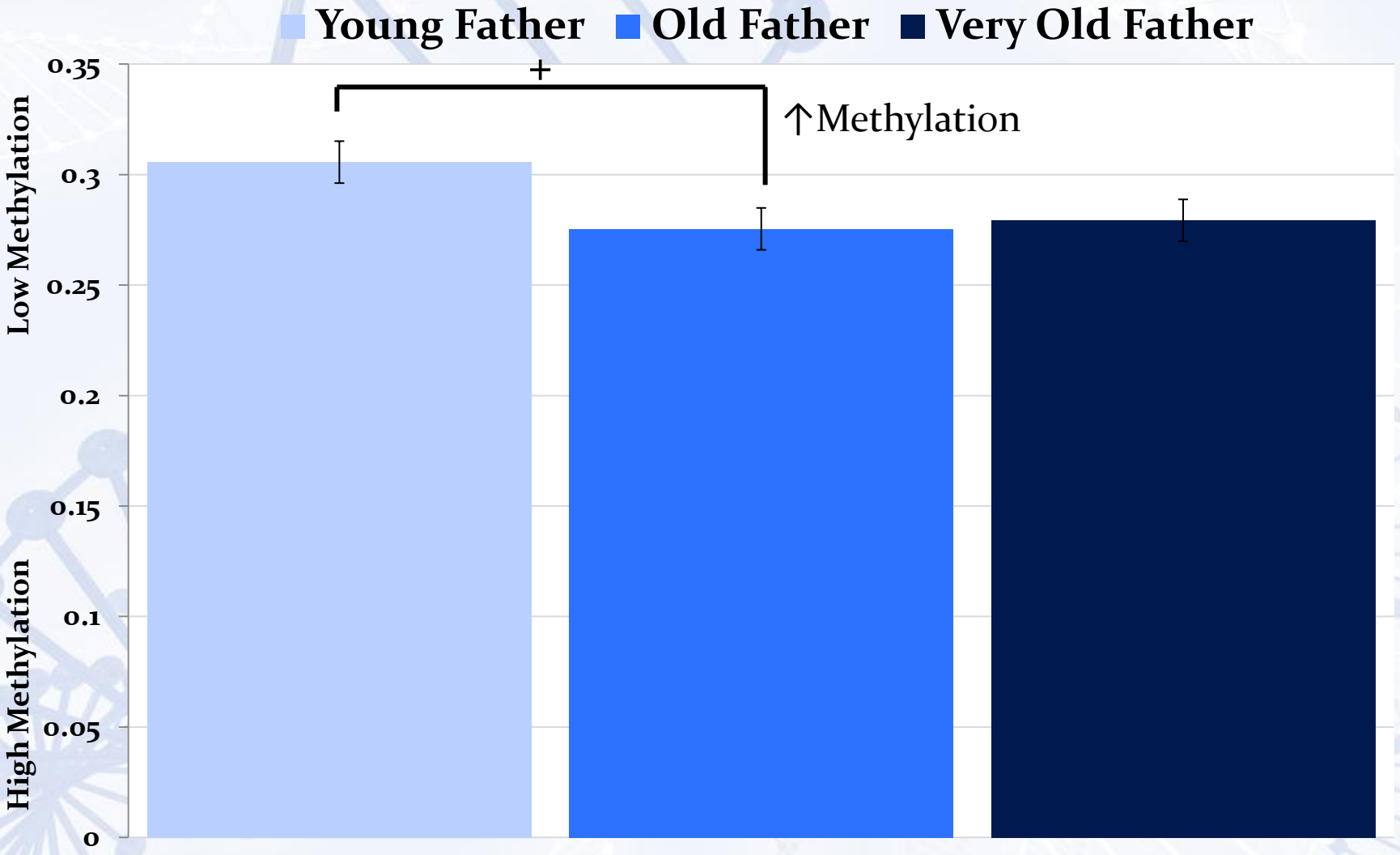
Global DNA Methylation
Spleen - Offspring



Global DNA Methylation
Spleen - Fathers



Global DNA Methylation Cerebellum - Offspring

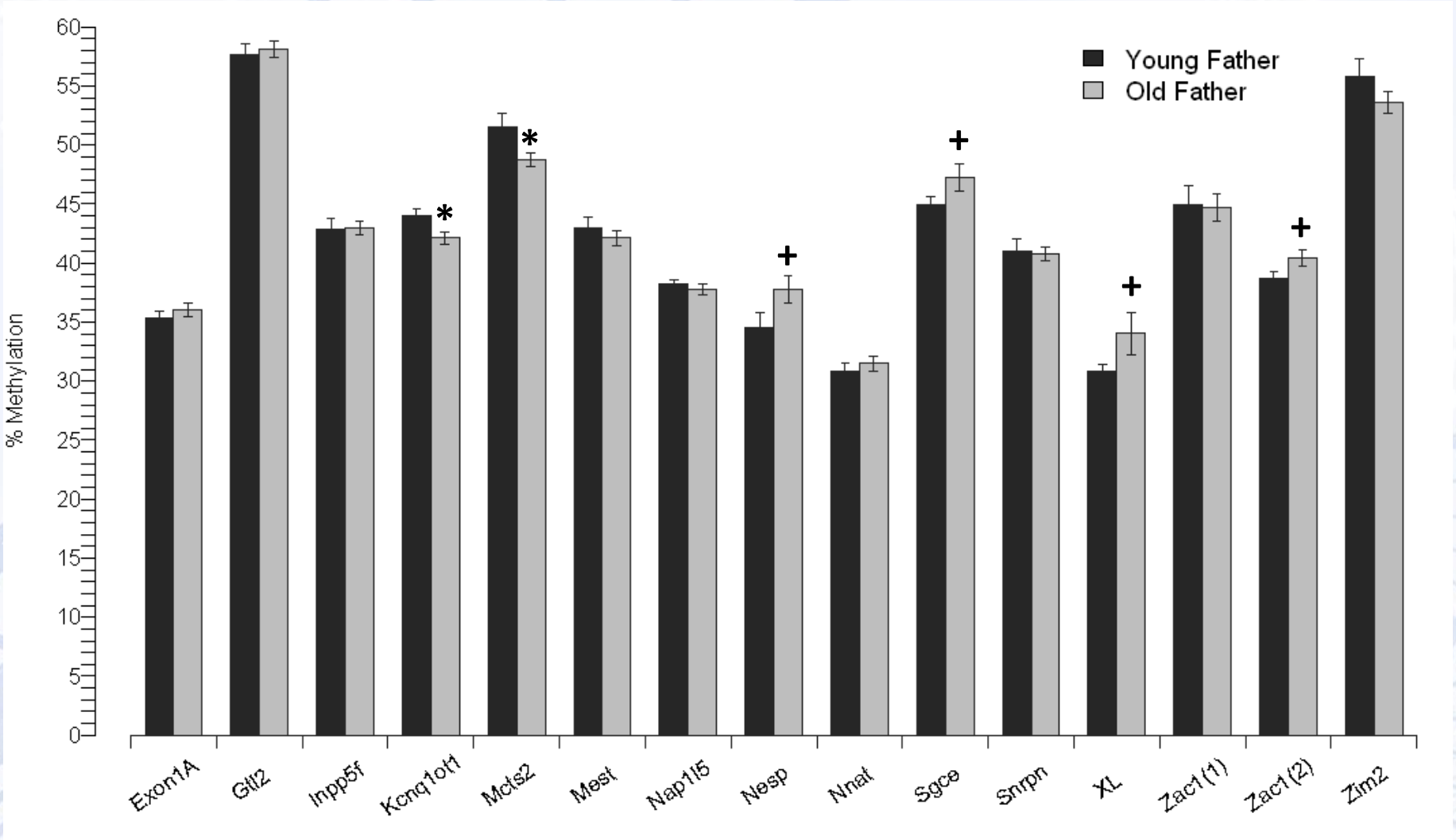


Imprinted Genes

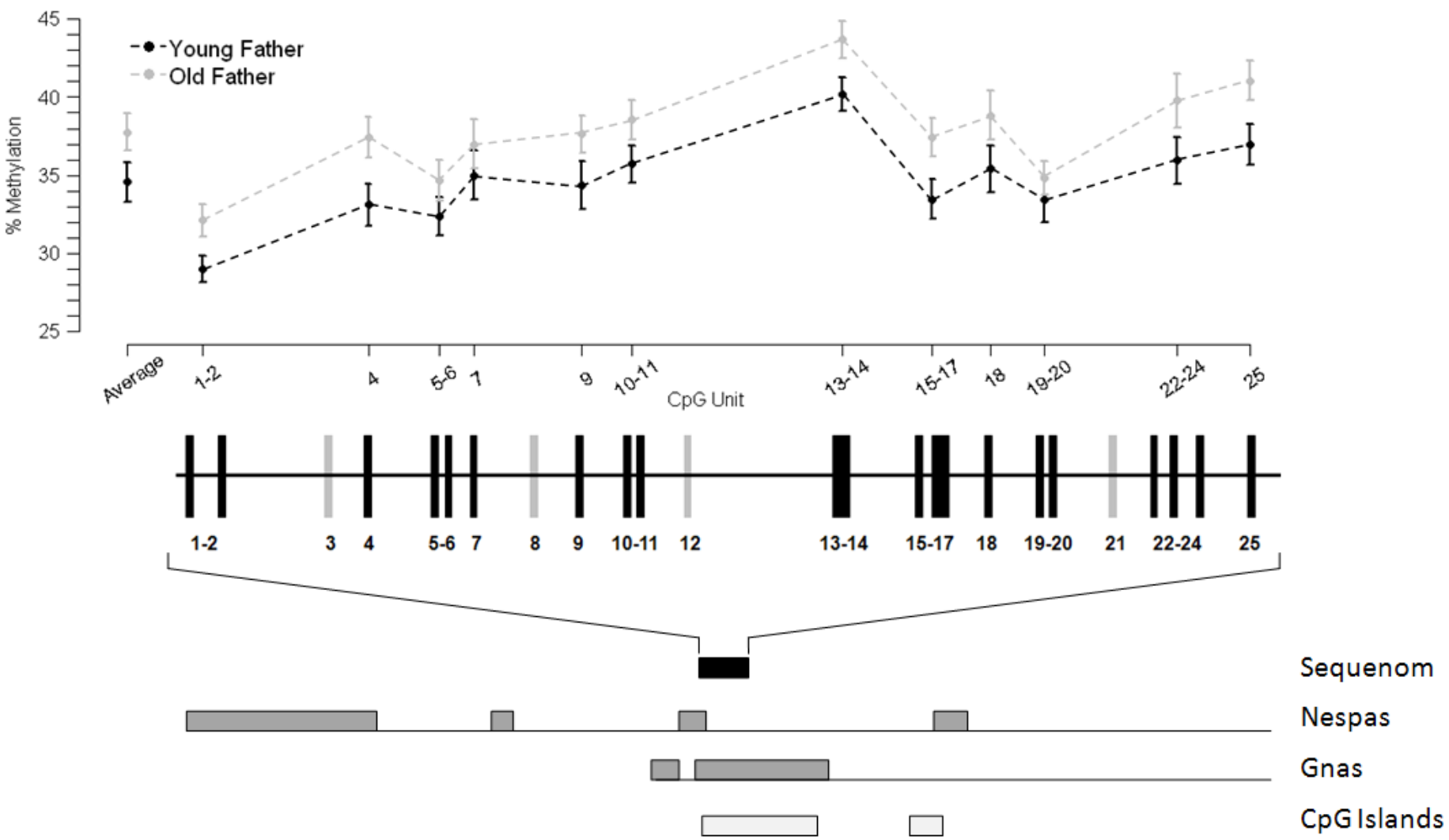
- Imprinting Dysfunction
- Epigenetic marks inherited from parents
- Genes expressed in parent of origin manner
- Focus on brain expressed imprinted gene
- Assays analysed using Sequenom EpiTyper

Gene	Expressed Allele
Cd81	Maternal
Gnas Exon 1A	Paternal
Gnas XL	Paternal
Grb10	Isoform Dependent
Gtl2	Maternal
Igf2	Paternal
Inpp5f	Paternal
Kcnq1ot1	Paternal
Mest	Paternal
Nap1l5	Paternal
Ndn	Paternal
Nesp	Maternal
Nnat	Paternal
Sgce	Paternal
Snrpn	Paternal
Ube3a	Maternal
Zac1	Paternal
Zim2	Maternal

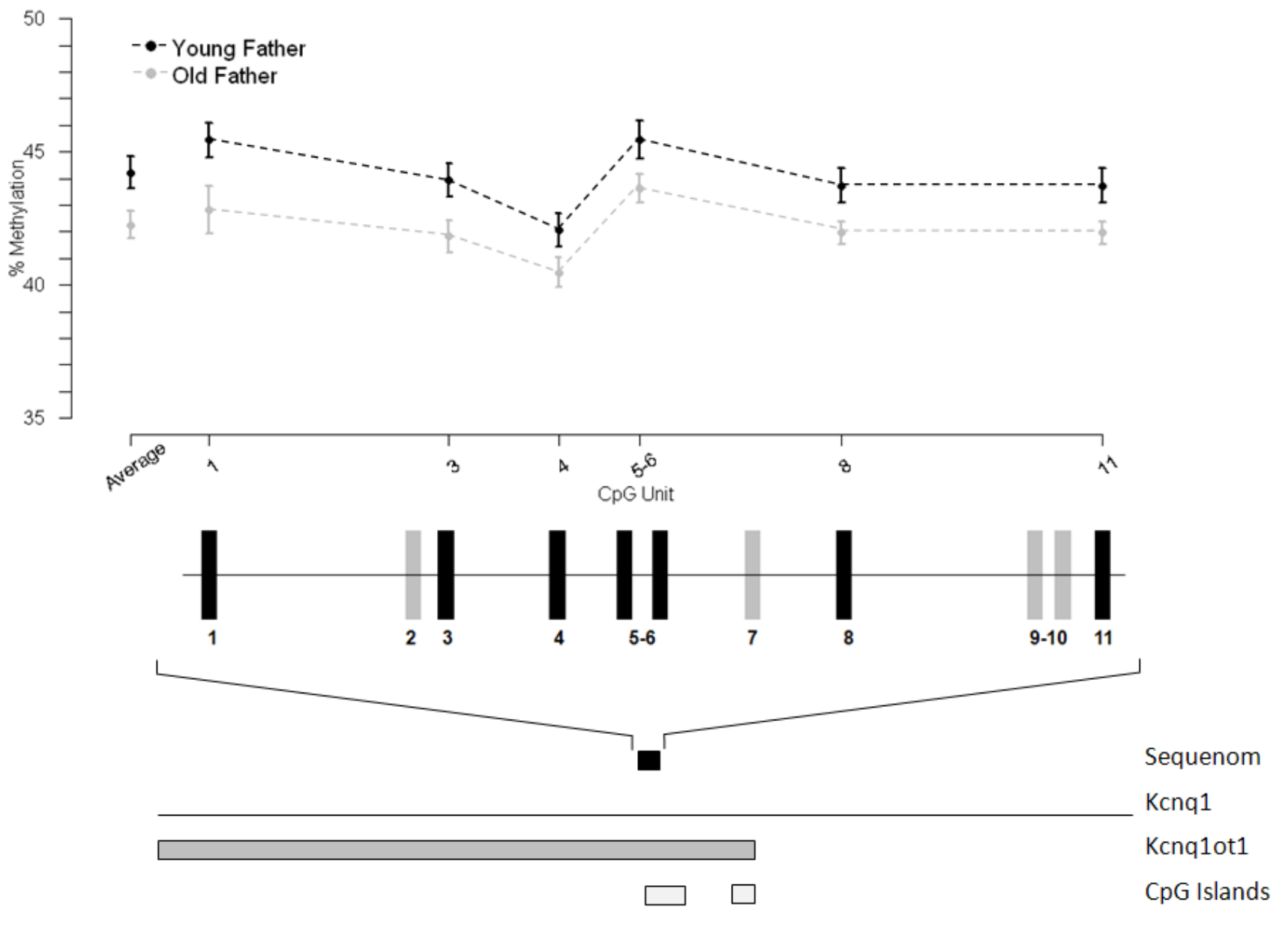
All DMRs - Cerebellum



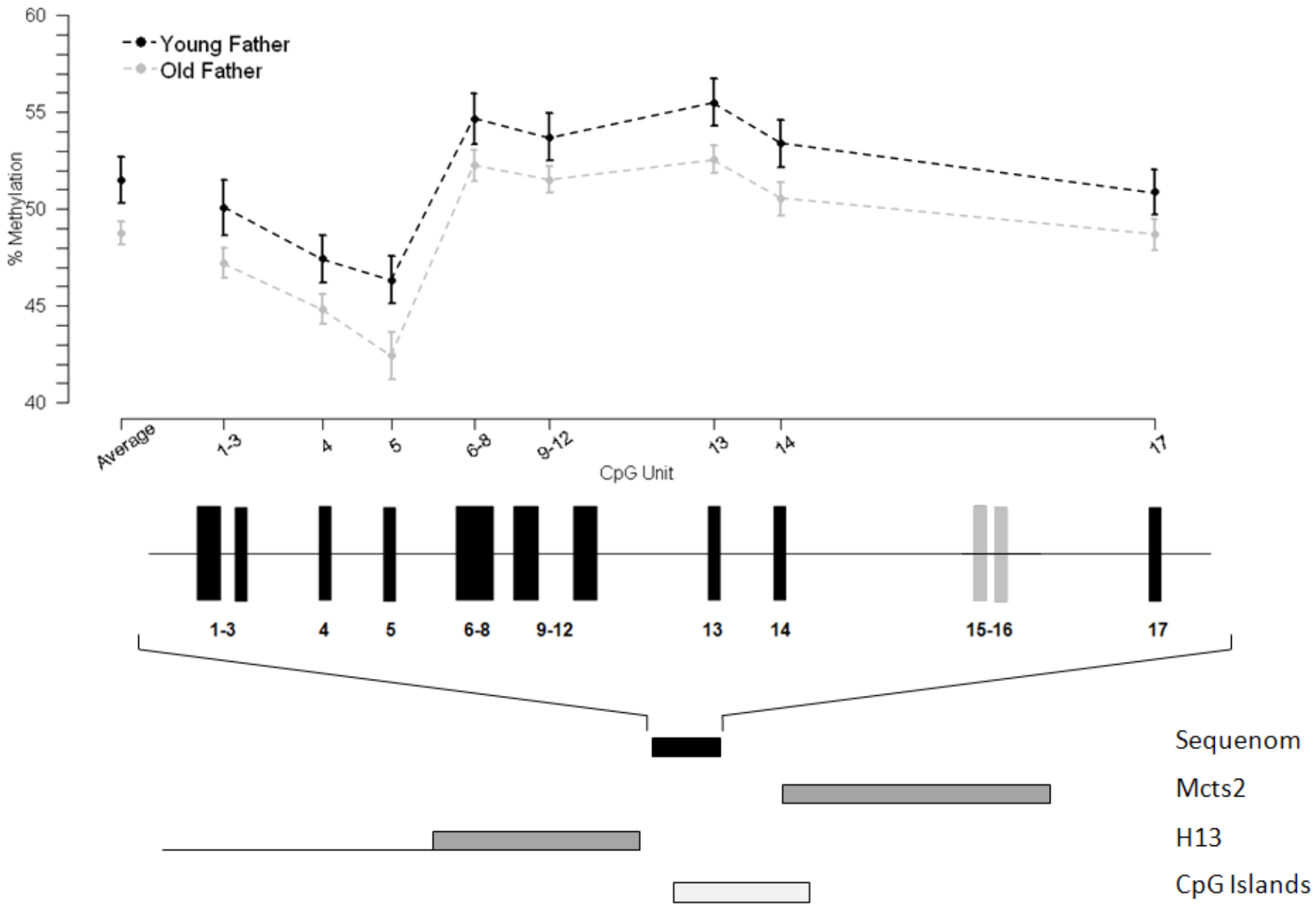
Nesp


Young Father
Old Father
% Methylation
45
40
35
30
25
Average
1-2
4
5-6
7
9
10-11
CpG Unit
13-14
15-17
18
19-20
22-24
25
1-2
3
4
5-6
7
8
9
10-11
12
13-14
15-17
18
19-20
21
22-24
25
Sequenom
Nespas
Gnas
CpG Islands

Kcnq1ot1

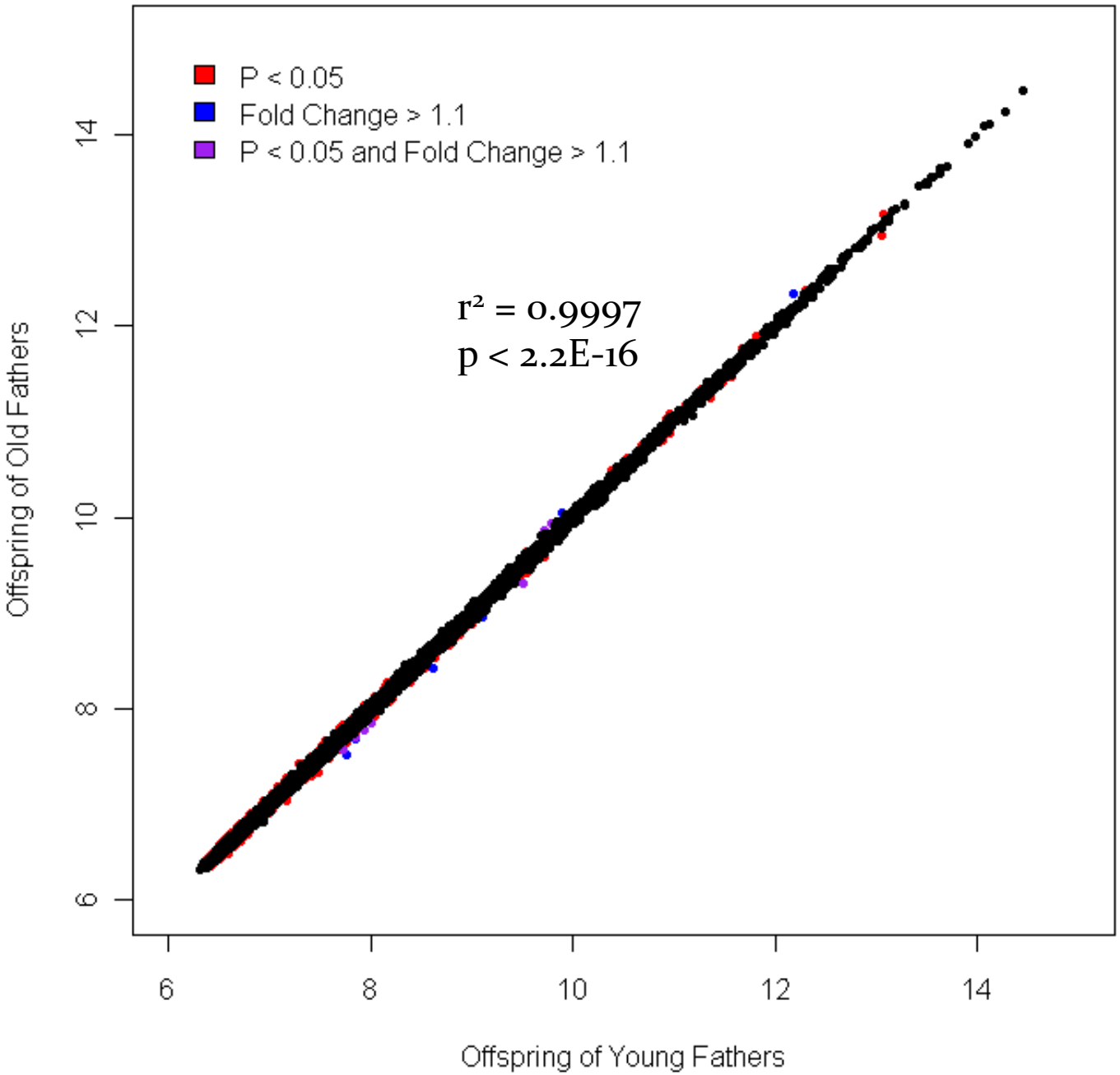

Young Father
Old Father
% Methylation
50
45
40
35
Average
1
3
4
5-6
8
11
CpG Unit
1
2
3
4
5-6
7
8
9-10
11
Sequenom
Kcnq1
Kcnq1ot1
CpG Islands

Mcts2

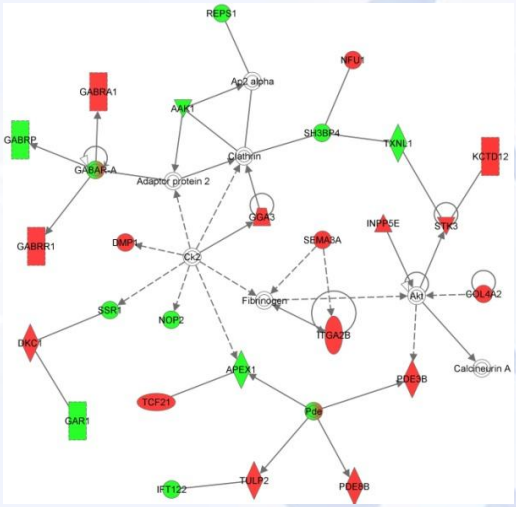

Young Father
Old Father
% Methylation
60
55
50
45
40
Average
1-3
4
5
6-8
9-12
13
14
17
CpG Unit
1-3
4
5
6-8
9-12
13
14
15-16
17
Sequenom
Mcts2
H13
CpG Islands

Expression Changes and Paternal Age

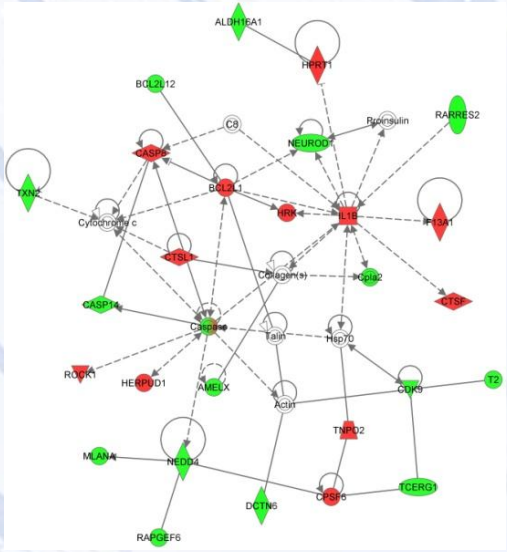
- Expression differences between offspring of young and old fathers
- RNA from frontal cortex of the offspring of young and old fathers
- Run on Illumina Mouse Ref8 V2
  - 25,600 RefSeq transcripts
  - over 19,100 unique genes
- Analysis in R
  - lumi


P < 0.05
Fold Change > 1.1
P < 0.05 and Fold Change > 1.1
r² = 0.9997
p < 2.2E-16
Offspring of Old Fathers
Offspring of Young Fathers
6
8
10
12
14

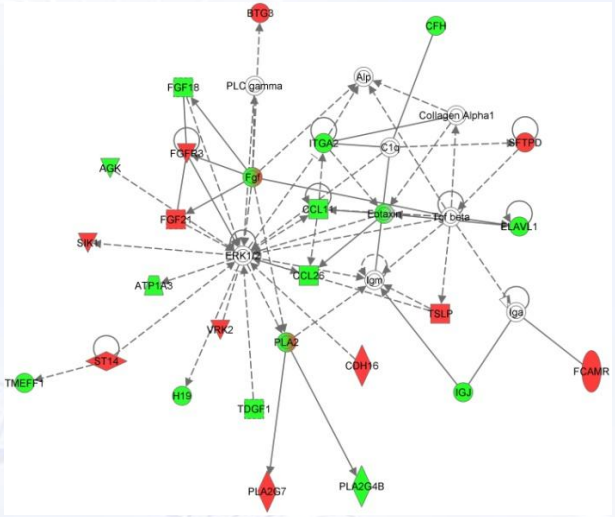
Cellular Assembly and Organization, Cellular Function and Maintenance Organismal Injury and Abnormalities



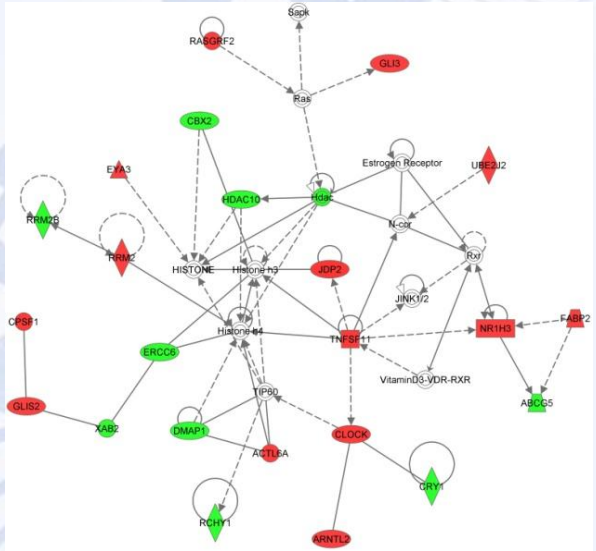
Cell Death, Cell Morphology, Cellular Function and Maintenance



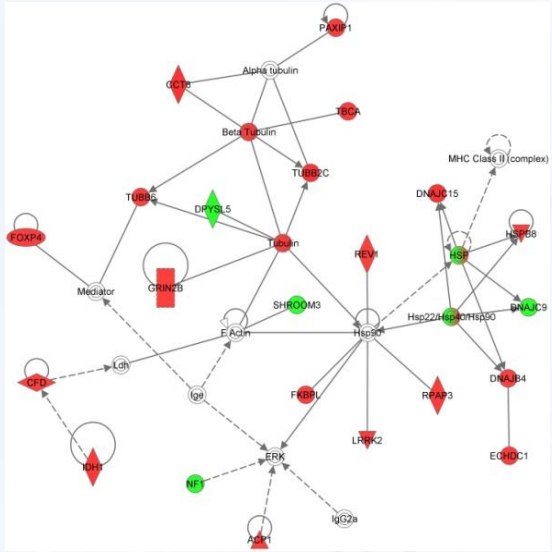
Inflammatory Response, Cellular Movement, Immunological Disease



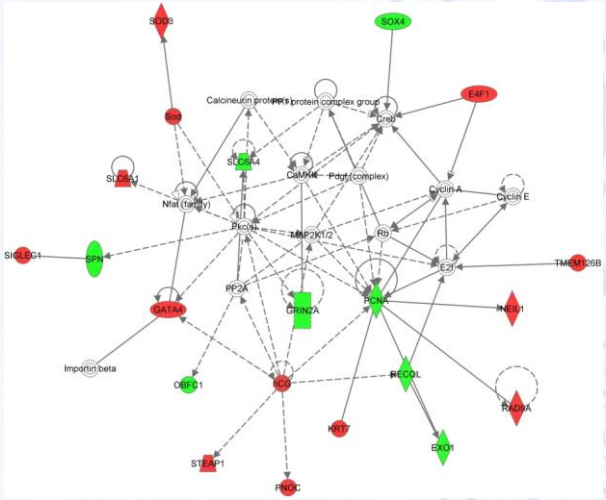
Gene Expression, Cell Cycle, Decreased Levels of Albumen



Post-Translational Modification, Cellular Movement, Hematopoiesis



DNA Replication, Recombination, and Repair, Cell-To-Cell Signalling and Interaction, Nervous System Development and Function

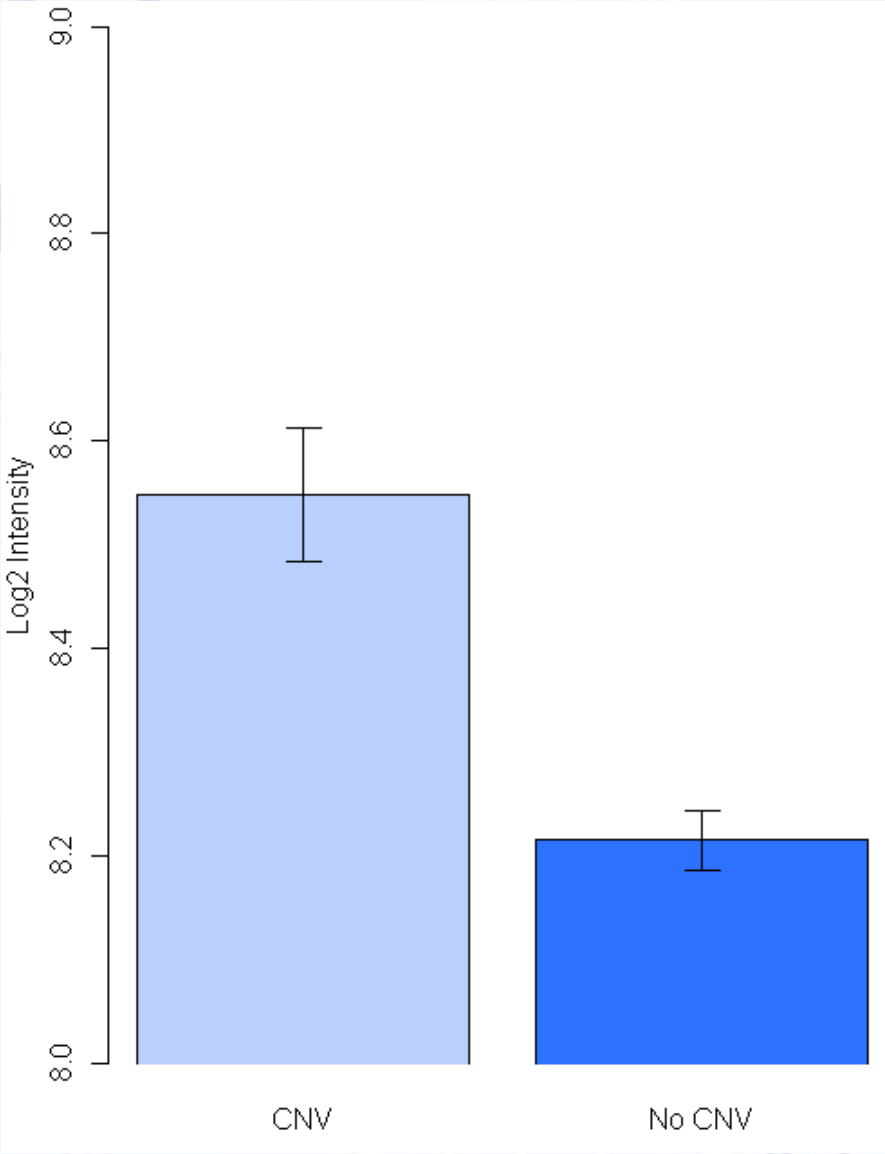


CNVs and Expression

- CNVs have been shown to have a strong effect on the expression of the genes they encompass
- CNVs have been shown to alter the expression of genes up to 0.5Mb away, although genes directly in the vicinity of a genomic alteration show a greater gene expression change

CNVs and Expression - *Ide*

- One CNV seen in six offspring
- CNV contains *Ide* which is covered by Illumina array
- Expression of *Ide* between CNV carriers and non-carriers significantly different (p = 0.002)
- Duplication carriers having higher expression with a fold change of 1.26



CNVs and Expression

- Most CNVs only seen in 1-3 individuals
- One sample t-test within R
  - Compares locus-specific gene expression in CNV carriers (designated mu) against the average expression for that gene across all individuals
- We restricted our analysis only to genes directly encompassed by the CNV
- 179 genes were identified that were affected by CNVs and had expression data
- 74% of genes in CNVs showed a significant gene expression difference between CNV carriers and non-carriers

Gene	Individuals with CNV	Fold Change	t-value	p-value	Direction	CNV Type
PITPNM1	1	1.28	6.73	1.57E-07	↓	Amplification
EDF1	1	1.24	-2.10	0.044	↓	Amplification
CORO1B	1	1.18	-8.10	3.82E-09	↓	Amplification
CHPF	1	1.18	4.85	3.26E-05	↓	Amplification
HBA-A1	1	1.15	-6.08	9.74E-07	↓	Deletion
CRK	1	1.14	3.97	3.93E-04	↑	Amplification
VEGFB	1	1.13	-4.27	1.73E-04	↓	Amplification
HIST1H2AO	3	1.13	-4.01	3.60E-04	↑	Amplification
ANXA11	2	1.13	-2.25	0.031	↓	Deletion
RPS6KB2	1	1.13	6.85	1.11E-07	↑	Amplification
TMEM198	1	1.12	-7.09	5.85E-08	↑	Amplification
TBC1D5	1	1.11	7.88	6.81E-09	↓	Amplification
PITPNM3	1	1.11	-4.37	1.29E-04	↓	Amplification
DBNDD1	1	1.11	7.37	2.66E-08	↑	Amplification
UBE3B	1	1.11	14.72	1.56E-15	↑	Amplification
MACROD1	1	1.10	2.72	0.011	↓	Amplification
A630095E13RIK	1	1.10	5.35	7.86E-06	↓	Amplification

Overall Conclusions

- Social and exploratory deficits with APA
- No difference in CNVs between groups
- Increased in global methylation with APA
- Several CpG units show methylation differences with APA
- Three DMRs show consistent methylation changes across region with APA
- No large changes in expression associated with APA
- IPA highlights pathways involved in the immune system and DNA replication, recombination and repair
- Majority of genes contained within CNVs show expression change

Acknowledgements

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