



Aggression in Children:
unraveling gene-environment
interplay to inform Treatment
and Intervention strategies



European Commission
Seventh Framework Programme

Epigenome-wide association study meta-analysis of aggressive behavior

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Netherlands Twin Register



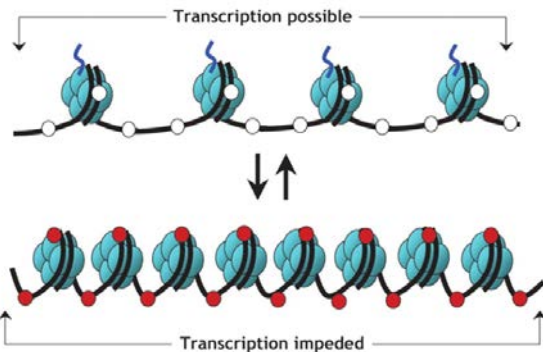
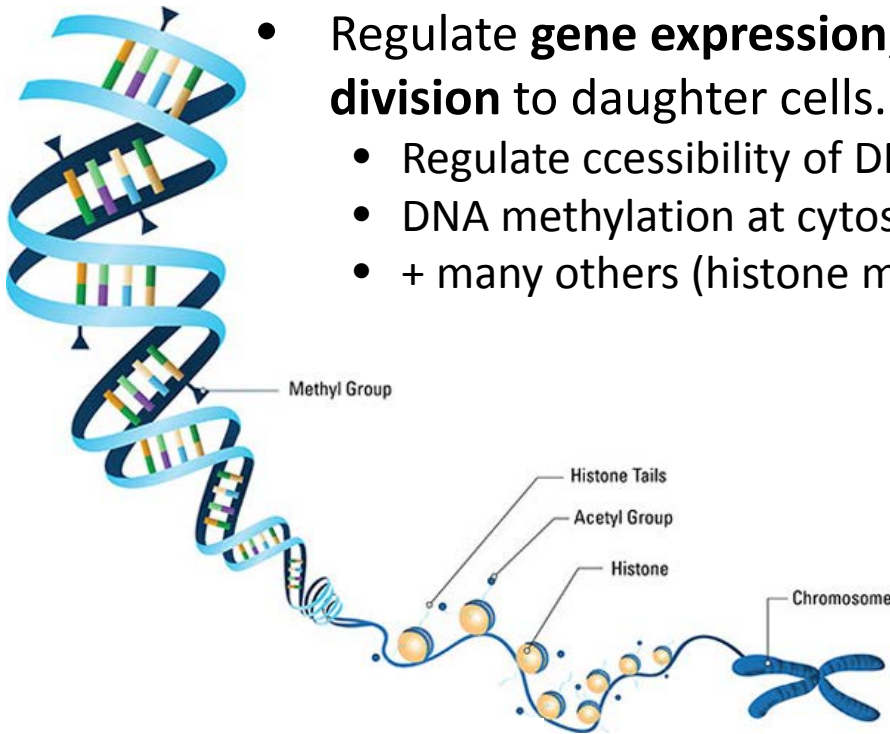
BGA Brisbane, June 23 2016



Epigenetic mechanisms



- Regulate **gene expression**, are **transmitted during cell division** to daughter cells.
 - Regulate accessibility of DNA to transcription
 - DNA methylation at cytosine-guanine nucleotides = **CpG sites**
 - + many others (histone modifications, non-coding RNA etc)



DNA methylation

CH₃ CH₃

 ACC**C**GTCAGATG**C**GATG

 TGG**G**CAGTCTAC**G**CTAC

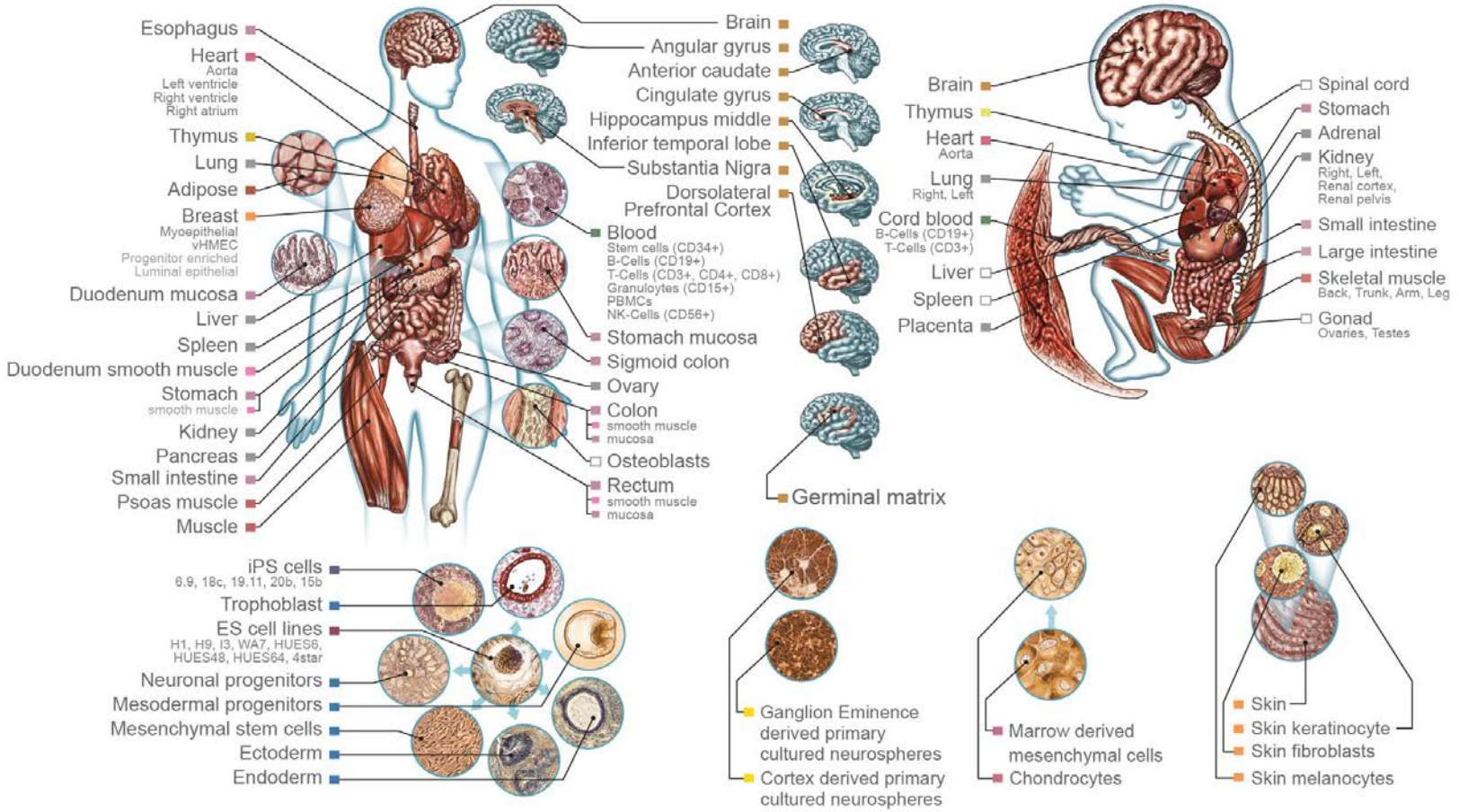
 CH₃ CH₃

CH₃=Methyl group

2



Each cell has its own *epigenome*



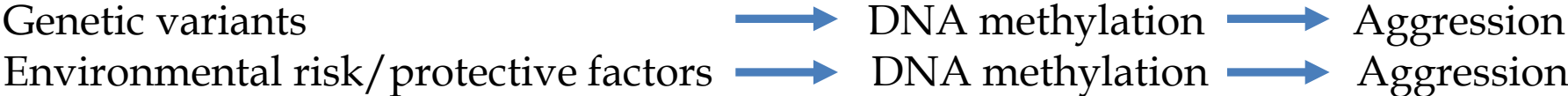
Kundaje, A, et al. "Integrative analysis of 111 reference human epigenomes." Nature 518.7539 (2015): 317-330.



DNA methylation level - Aggression



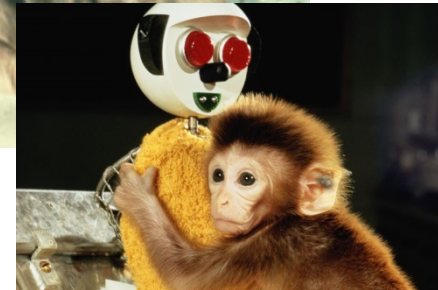
Mean heritability ~19%, Illumina 450k, whole blood (refs 1,2)
Environmental influences affecting DNA methylation:
(maternal) smoking and diet, (early) life conditions



1. McRae et al Genome Biology (2014).
2. van Dongen J. et al. Nature Communications (2016).



DNA methylation - Aggression



Surrogate mother+ pear-rearing condition
Inadequate social skills, highly aggressive, increased voluntary alcohol consumption

Provencal et al. **The Signature of Maternal Rearing in the Methylome in Rhesus Macaque Prefrontal Cortex and T Cells.** *The Journal of Neuroscience* 32.44 (2012): 15626-15642.



DNA methylation - Aggression



Human studies:

Provençal, Nadine, et al. "Differential DNA methylation regions in cytokine and transcription factor genomic loci associate with childhood physical aggression." *PLoS one* 8.8 (2013): e71691.

Guillemin, Claire, et al. "DNA methylation signature of childhood chronic physical aggression in T cells of both men and women." *PloS one* 9.1 (2014): e86822.

Twin Research and Human Genetics

Twin Research and Human Genetics / Volume 18 / Issue 06 / December 2015, pp 686-698
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DOI: <http://dx.doi.org/10.1017/thg.2015.74> (About DOI), Published online: 28 October 2015

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PDF (528 KB) HTML (795 KB) Supplementary Materials

Previous Abstract

Altmetric 12

SPECIAL SECTION: Epigenetics and Twin Research

Epigenome-Wide Association Study of Aggressive Behavior

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N= 2029, methylation (IL450k) blood, mean age= 36

EWAS= Epigenome-wide Association Study

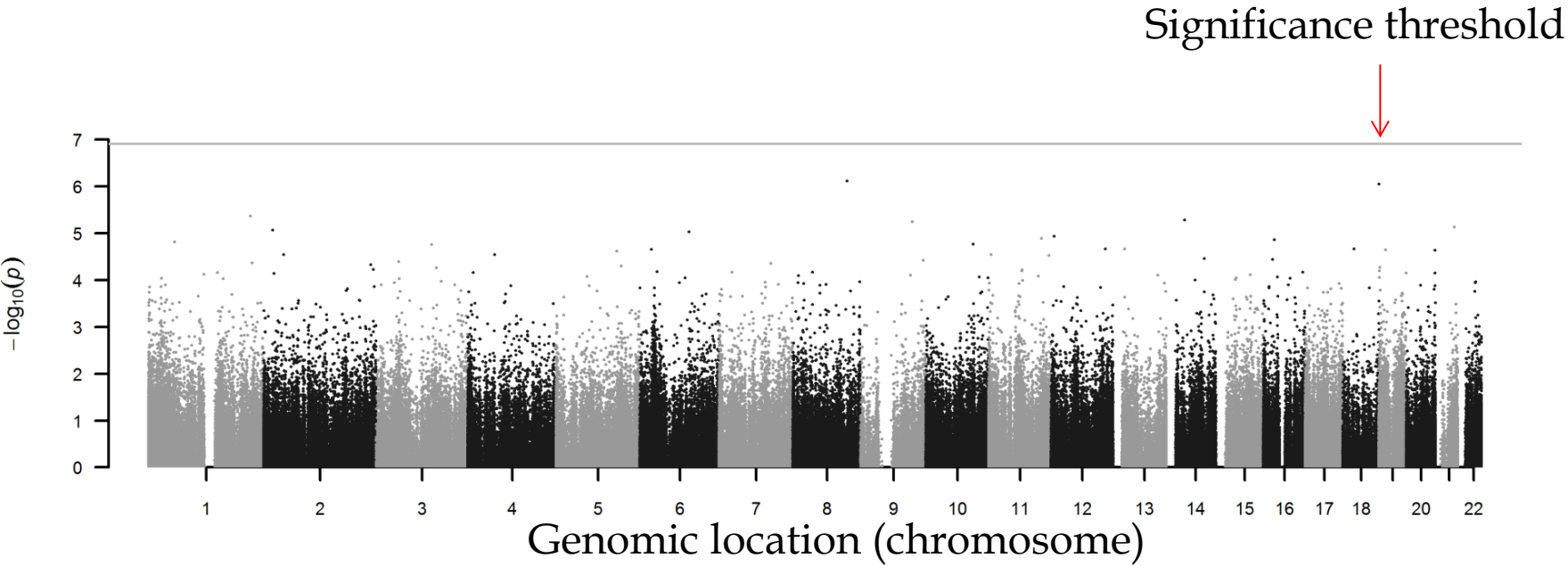




Epigenome-wide association study in NTR(N=2029)



High-ranking sites: significant enrichment of central nervous system Gene Ontologies





EWAS meta-analysis Aggression



- **Invitation of cohorts (started January 29 2016)**
- **N cohorts invited: 31**
 - Positive response: 10
 - Negative response: 19
 - Reason: no aggression data=15
 - Reason: no 450k methylation data=3
 - Reason unknown=1
 - Awaiting response to invitation/under consideration: 2 cohorts
- **Detailed analysis plan and exemplary analysis scripts**
- **Summary statistics and cohort information shared through SFTP server**



Participating cohorts – June 18 2016



Study	Study	tissue	Aggression data	N
NTR	Netherlands Twin Register	PWB	ASEBA Adult self-report (ASR) aggression scale	2029
FT12	FinnTwin 12	PWB	Multidimensional Peer Nomination Inventory (MNPI) – aggressive behavior	757
GenR	Generation R	UCB	Child Behavior Checklist (CBCL) 1½ – 5 years Aggressive Behavior scale	806
LLD	LifeLines-DEEP	PWB	“I am known for being short-tempered and irritable” (NEO personality)	683
BSGS	Brisbane Systems Genetic Study	PWB	Buss and Perry aggression questionnaire	?
ALSPAC	Avon Longitudinal Study of Parents and Children	PWB	SQD conduct problem scale	~900
NFBC1966	Northern Finnish Birth Cohort 1966	PWB	“I lose my temper more quickly than most people”	780
NFBC1986	Northern Finnish Birth Cohort 1986	PWB	ASEBA Youth self-report (YSR) aggression scale	580
Gecko	Groningen Expert Center for Kids with Obesity	?	SDQ conduct problem scale	?
INMA	Environment and Childhood Project	PWB	SDQ conduct problem scale	200

PWB= peripheral whole blood

UCB = umbilical cord blood

<http://www.action-euproject.eu>

results uploaded (NTR,FT12,GenR, LLD) :

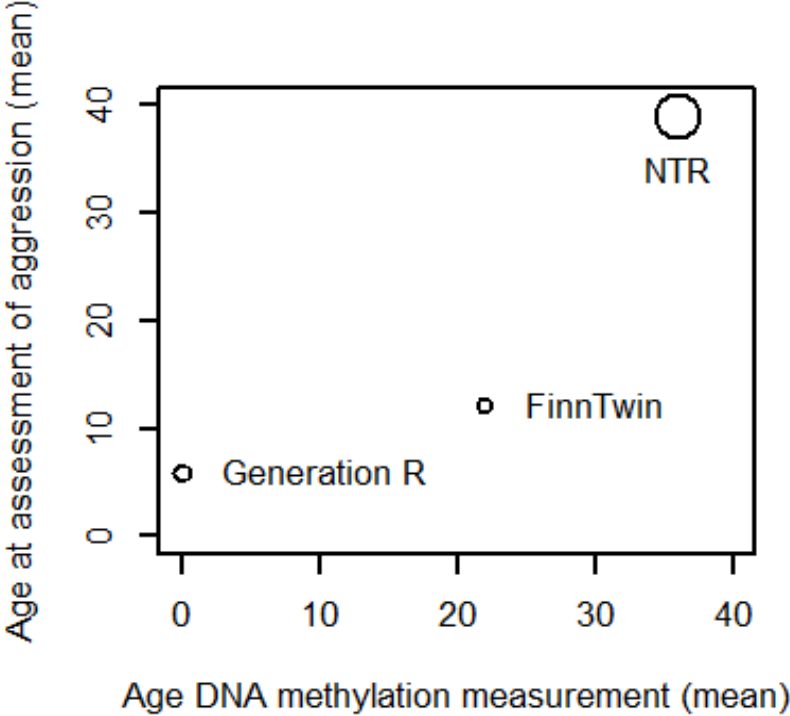
4275

Expected N :

>7000



Meta-analysis first 3 aggression cohorts (N=3622)



P-value based fixed effects meta-analysis, R package gap

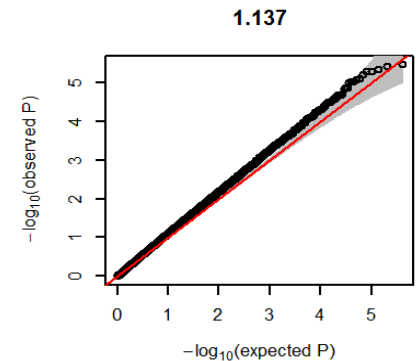
Cohort	survey	N	Age @DNA sampling	Years methylation - phenotype, (mean, min:max)
Netherlands Twin Register	ASR	2059	36.4	-2.6 (-10: 8)
Generation R	CBCL	806	birth	5,9 (5,2: 8,3)
FinnTwin 12	MNPI	757	22.43	10.43 (9:13)



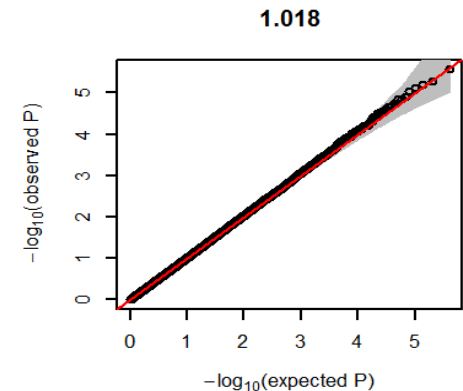
Results - Genome-wide Bonferroni threshold or FDR <0.05



- **Model 1 - no adjustment for BMI or smoking**
- Netherlands Twin Register: 0
- FinnTwin: 0
- Generation R (umbilical cord blood DNA):
1 CpG site (*IL1B*, $p= 6.60E-08$)
- Meta-analysis (Total N subjects=3622): 0



- **Model 2 -BMI and smoking included as covariates**
- Netherlands Twin Register: 0
- FinnTwin: 0
- Generation R: 0
- Meta-analysis (Total N subjects=3622): 0

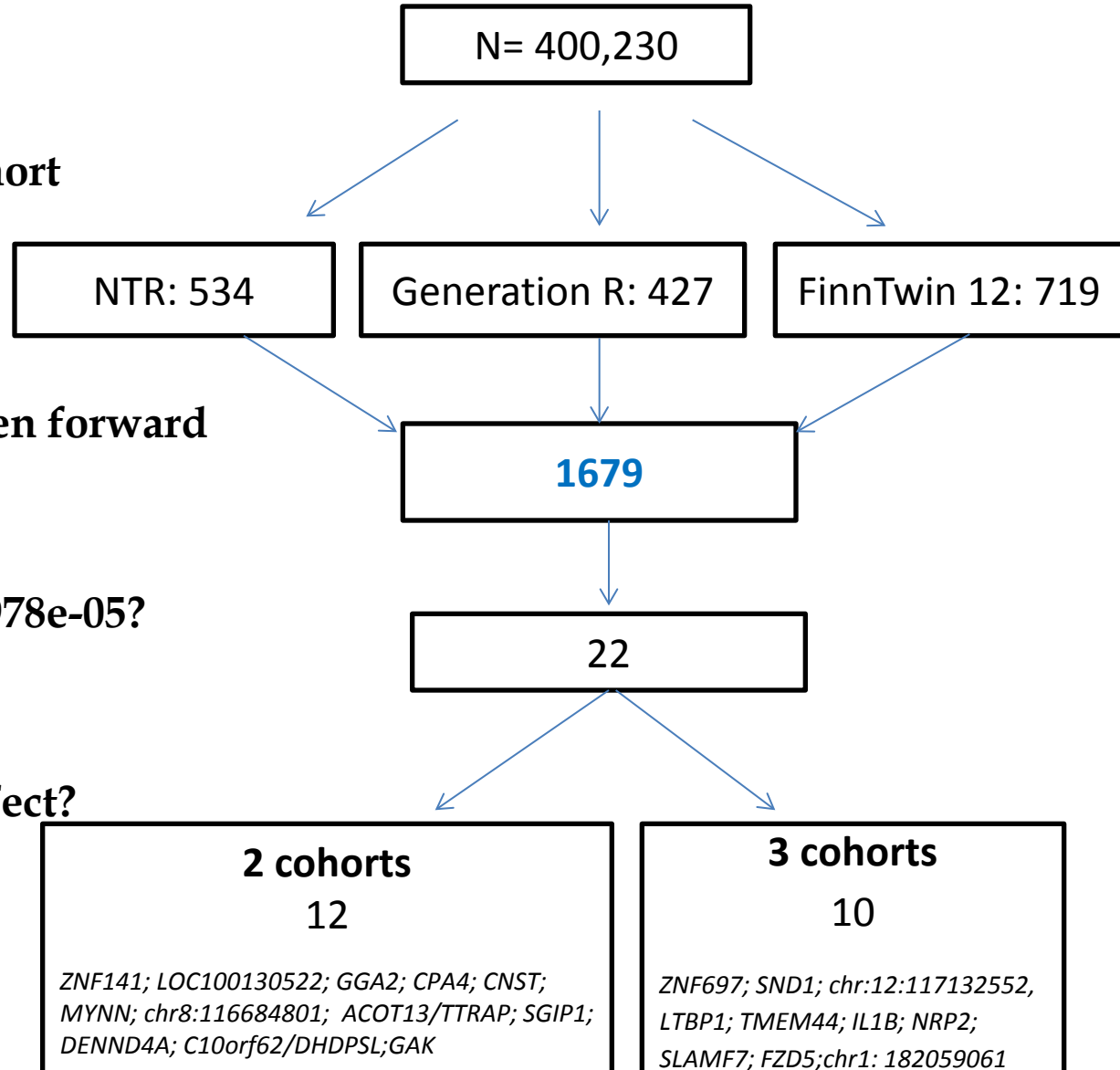




Exploratory meta-analysis

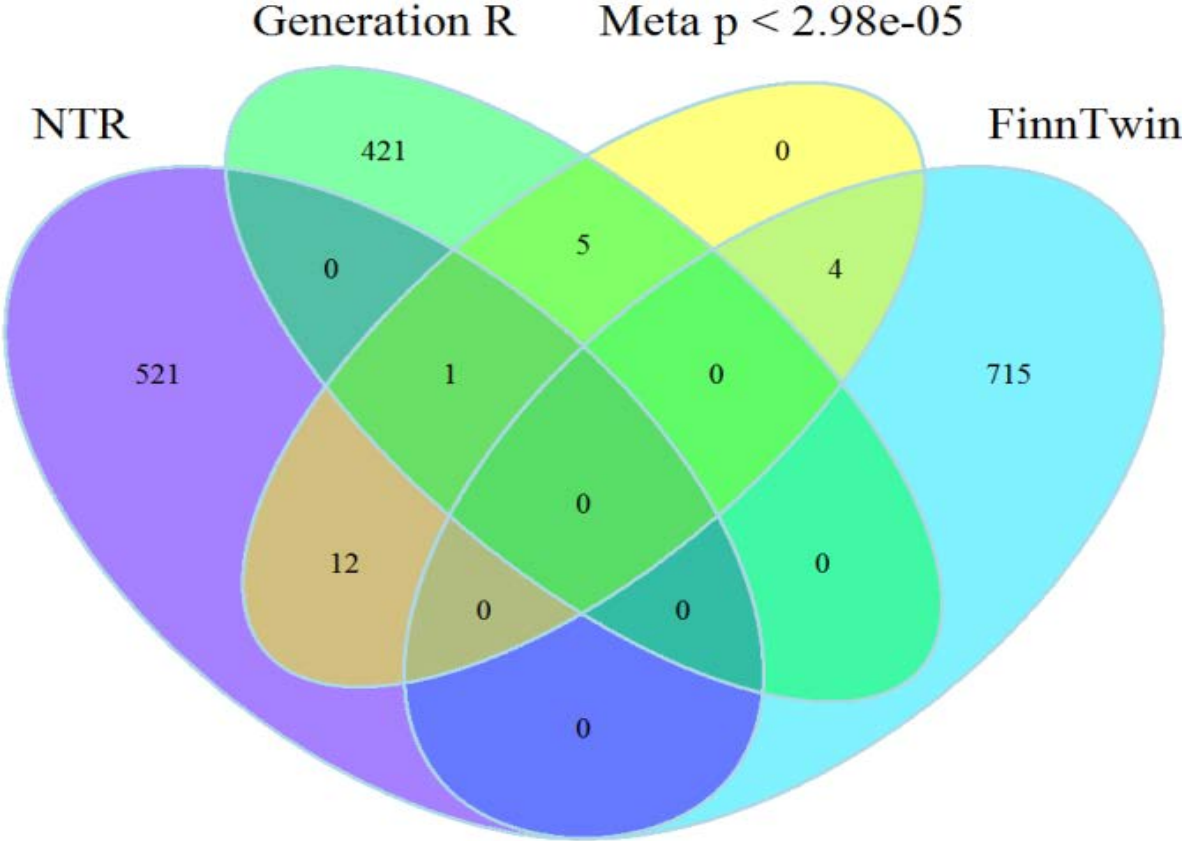


- All sites
- $P < 1 \times 10^{-3}$ in any cohort
- Promising CpGs taken forward to meta-analysis
- Meta-analysis $p < 2.978e-05?$ (0.05/1679)
- Same direction of effect?





Overlap of 1679 promising probes ($p < 0.001$)





Some highlights - sites from exploratory analysis with same direction of effect in all 3 cohorts



cgid	Gene	Literature – gene function/ associated phenotypes	refs
cg18635064	<i>IL1B</i>	Cytokine; mediator of the acute phase response. Cytokines have previously been implicated in aggression.	Provençal, N. et al PLoS ONE 8, e69481 (2013)
cg24680162	<i>ZNF697</i>	neuronal survival, differentiation, and synaptic plasticity.	Chan Genomics Data 2015
cg05068777	<i>SND1</i>	autism; fragile X syndrome; schizophrenia	Holt European Journal of Human Genetics (2010) 18, 1013–1019; Bittel Genetics in Medicine (2007) 9, 464–472; Fromer Nature 2014
cg14260773	<i>LTBP1</i>	alcohol consumption. TGF-beta signaling pathway : Alzheimer’s disease and schizophrenia, vascular development and vascular disease	Pei 2012 PLOS ONE, Pannu AJMG 2005
cg18017245	<i>TMEM44</i>	Oculo auriculo vertebral spectrum; deletion of the region: autism, intellectual disability, diverse psychiatric disorders , obesity	Guida 2015 American Journal of Medical Genetics Part A; Biamino 2016 American Journal of Medical Genetics Part B: Neuropsychiatric Genetics; Yang Virology Journal 2011:80 Volume 171, Issue 2, pages 290–299, March 2016
cg05348875	<i>NRP2</i>	Cg05348875 methylation associated with prenatal maternal smoking . Gene associated with autism, mental retardation.	meta-analysis Joubert AJHG 2016
cg23844325	<i>SLAMF7</i>	Gene methylation associated with posttraumatic stress disorder; alcohol dependence in mouse model	Uddin Proc Natl Acad Sci U S A. 2010
cg01201797	<i>FZD5</i>	Wnt receptor. Wnt signalling is involved in synapse formation	see: Okerlund Journal of Neurodevelopmental Disorders 2011 3:9083



Next steps



- Extra cohorts to join: Brisbane Systems Genetic Study, ALSPAC, NFBC1966 and NFBC1986, Gecko, INMA + 2 more under consideration
- Still welcoming additional cohorts
- Follow-up analyses integrating methylation and gene expression data (RNA-seq and/or microarray)
- We are looking for cross-tissue replication cohort (cohort with **aggression and methylation in e.g. brain**, buccal, skin etc)

- We are working on a similar project for subjective wellbeing
- **Suggestions for cohorts with IL450k/IL850k methylation and aggression or wellbeing data welcome!**

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Acknowledgements



Khadeeja Ismail
Rosa Mulder
Marc Jan Bonder
Bart M. L. Baselmans
Michel G. Nivard
Rick Jansen
Lannie Ligthart
Miina Ollikainen
Eero Vuoksima
Lude Franke
Henning W. Tiemeier
Jaakko Kaprio
Meike Bartels
Dorret I. Boomsma



This study was funded by:
ACTION. ACTION receives funding from the European Union Seventh Framework Program (FP7/2007-2013)
BBRMI-NL-financed BIOS Consortium (NWO 184.021.007)



Thank you for your attention

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