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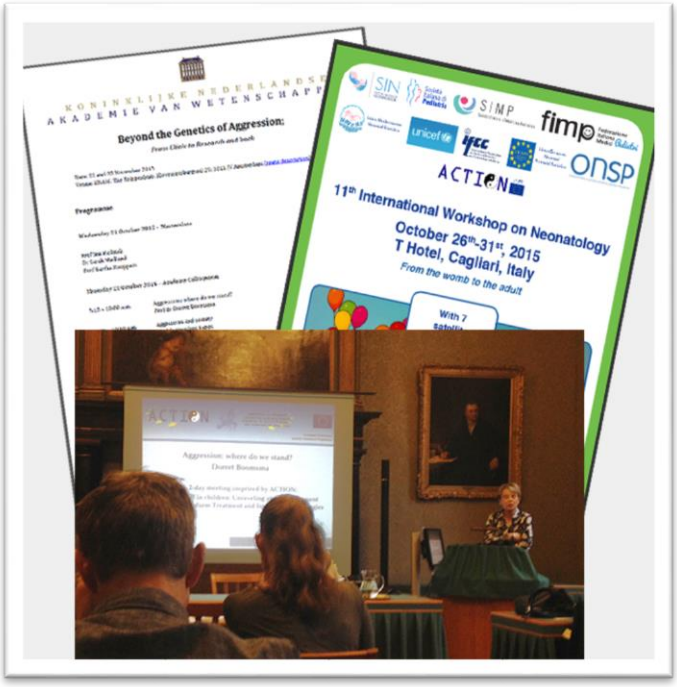
The ACTION Newsletter

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

N° 1, December 2015

In this issue



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ACTION

*To obtain an understanding of why children differ from each in other in aggressive behaviors, the ACTION consortium was established. ACTION stands for "**A**ggression in **C**hildren: Unravelling gene-environment interplay to inform **T**reatment and **I**nterventi**ON** strategies". Twelve partners - from the Scandinavian countries (Finland and Sweden), the UK, the Netherlands, and Italy - together with scientists from the USA and Australia will collaborate to solve the questions and needs indicated by clinicians, social workers and parents and teachers of children with aggression problems.*

In the ACTION research program the world's largest registers with data from young twins collaborate to elucidate the way in which genes covary and interact with environmental risk and protective factors. Next levels of understanding the pathways from genotype to behavior include epigenetics and biomarkers based on metabolomics approaches, as well as a better understanding of what constitutes childhood aggression: do we ask parents, teachers, children themselves about their problems, and what questions should we ask them?

Two meetings where all collaborators met were organized in Amsterdam. During the second meeting the first results obtained in the ACTION project were presented by PhD students, postdocs and PIs. These included high estimates for the heritability of aggression, the first genome-wide epigenetics study of aggression in adults and the first experiences of collecting biological samples from twins and patients for this project.

Aggression inflicts a huge burden on affected children, their families, and society. It is estimated that between 2 and 16% of children suffer from aggression problems. Childhood aggression tends to continue into adulthood. There is a huge need for more personalized approaches, which requires insight into the heterogeneity and the mechanisms underlying aggression and its associated comorbidities. The FP7-ACTION project aims to contribute to knowledge that will help children, their families, teachers and society at large.

Prof. Dorret Boomsma
ACTION Project Coordinator
PhD, Professor of Biological Psychology,
Head of Department of Biological Psychology
VU University Amsterdam

2nd Project Meeting

A 3-day ACTION meeting was organized at the Royal Netherlands Academy of Science (KNAW) in Amsterdam on October 21 – 23. The meeting received financial and organizational support from the KNAW. Members of executive committee, cohort leaders, PhD and Postdocs, Scientific and Ethic Advisors were present.


KONINKLIJKE NEDERLANDSE AKADEMIE VAN WETENSCHAPPEN

Beyond the Genetics of Aggression;

From Clinic to Research and back

Programme

On October 21st the meeting started with a masterclass (Beyond the Genetics of Aggression; From Clinic to Research and Back) for PhD students, postdocs and employees working on ACTION aggression research. During the master class some presentations were given by the participants, who received questions and comments from ACTION members (Dr. Sarah Medland, Prof. Meike Bartels and Prof. Dorret Boomsma), from the Scientific Advisory Board (Prof. James Hudziak) and from the Ethic Advisory Board (Prof. Bartha Knoppers).

2nd Project meeting, Amstedam.
Full photogallery available on ACTION website

Thursday October 22nd: Aggression: Progress and Next Steps

On Thursday, the meeting focused on a series of updates and progress reports from the Work Packages:

- "Aggression: where do we stand?", Prof. Dorret Boomsma (WP 1)
- "Aggression and society", Prof. Vassilios Fanos (WP 7)
- "Social outcomes of aggression", Prof. Paul Lichtenstein (WP 2)
- "Needs and questions among clinicians", Prof. Robert Vermeiren (WP 2)
- "Genetic Epidemiology of Aggression", Prof. Meike Bartels (WP 3 and 4)
- "The aggression phenotype", Dr. Gitta Lubke (WP 2, 3, 4)
- "Biomarkers: Metabolomics and aggression", Prof. Thomas Hankemeier (WP 5)

Friday October 23rd

The meeting started with presentations of PhD students and postdocs who are employed on ACTION.

- "Genome-wide polygenic scores: options for phenome analyses", Eva Krapohl
- "Metabolomics and aggression", Fiona Hagenbeek
- "Predicting aggressive behavior from genetic risk for ADHD and ASD", Dr. Lucía Colodro Conde
- "Association of childhood aggression with metabolic polygenic scores", Patrick Miller
- "Biomarker collection in clinical practice", Peter Roetman
- "Identifying ACTION intersections of measures and informants", Anne Hendriks
- "Epigenetics and Aggression", Dr. Jenny van Dongen (presented by Prof. Boomsma due to Dr. van Dongen absence)

The **ACTION Newsletter is proud to present these contributions**. The next pages will focus on some of the PhDs and postdocs in ACTION and their research projects.

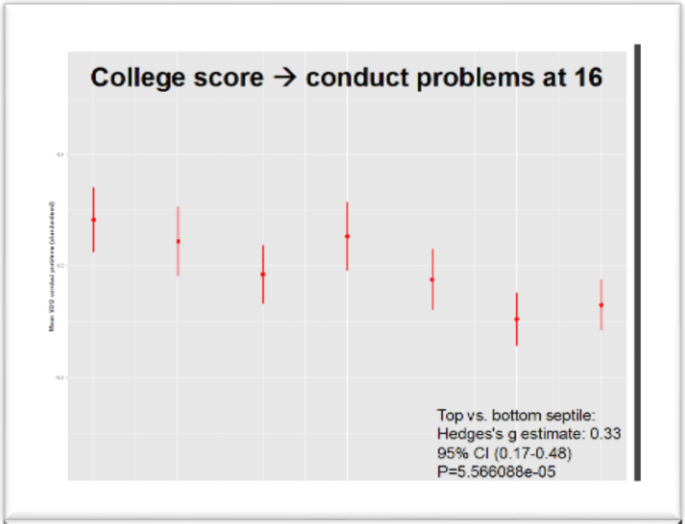
Genome-wide polygenic scores: options for phenome analyses

Eva Krapohl, King's College London

One of the ultimate aims of our research is early individual phenotype prediction. As a tentative step in this direction, we tested whether genomewide polygenic scores (GPS), based on current GWAS, predict phenotypic variation in the adolescent population.

In the presence of robust evidence for polygenicity and pleiotropy of complex traits, we wanted to conduct analyses that go beyond testing genetic effects on a single trait or of a single genetic variant: A 'phenome-wide analyses of genome-wide polygenic scores'.

Specifically, we tested profiles of associations between 13 polygenic scores from current GWAS for psychiatric disorders and cognitive traits as they relate to 50 behavioral traits in a UK-representative sample of 3000 16-year-olds. We included 50 phenotypes measured at the end of compulsory schooling at age 16; from the domains of psychopathology, personality, cognitive abilities and educational achievement. Conduct problems were measures by the parent-reported SDQ conduct problems scale.



College polygenic sore predicts conduct problems at age 16. Full article accessible here: Krapohl, E. et al. Phenome-wide analysis of genome-wide polygenic scores. Molecular Psychiatry (2015). doi:10.1038/mp.2015.126

Apart from BMI and height, polygenic score for College, based on the binary measure of attending college or not, showed the strongest profile of association with phenotypes at age 16. Interestingly and relevant to ACTION, negative associations of college GPS emerged for total behavior problems and for conduct problems explaining up to 1% of the variance.

Despite the modest effect sizes of current GPS, quantile analyses illustrate the ability to stratify individuals by GPS and opportunities for research. For example, the highest and lowest septiles for the education GPS yielded a ~0.3 s.d. difference in mean conduct problems at age 16.

Conclusions, comments and future activities.

With the new SSGAC and UKBiobank education GWASs coming out, predicting children's aggressive behaviour with education polygenic scores might be a promising strategy. And arguably, there is untapped research potential for selecting or stratifying individuals by polygenic score quantiles.

Merging data from different ACTION cohorts will allow for more powerful analyses than conducting meta-analyses. The first data release of the Haplotype Reference Consortium (HRC) yields more reliable

imputation especially for low frequency variants; and it aids in harmonizing samples genotyped on different platforms.

ACTION analyses proposed during talk:

Aim: Phenotype harmonisation by investigating homogeneity/pleiotropy of aggression measures across ACTION cohorts.

- Estimate the genetic correlation between different aggression measures across different ACTION cohorts. Ideally, by running GREML/GCTA on pooled data. Or else, meta-analyzing summary statistics using LDscore regression correlations (this option is less powerful for sample size in the region of ACTION cohorts).

Aim: Investigate genetic link between education and aggressive behavior.

- Conduct bivariate twin analyses of educational achievement and aggressive behaviour.
- Conduct bivariate cross-cohort GREML/GCTA of educational achievement and aggressive behaviour.
- In contrast to the twin method, GCTA is not limited to decomposing phenotypes that can vary within families. Therefore, conduct bivariate GCTA analyses of between between-family (aka "environmental") factors such as parental education/socio-economic status and children's aggressive behavior.
- Population-based analyses of the education-aggression link in the Swedish population could get us closer to causal inference.

Aim: Predict individual-specific aggressive behavior using education polygenic scores

- Predict aggression in ACTION cohorts using education polygenic scores (Using the GWAS summary statistics from the second round of the SSGAC or the UKBiobank education GWASs).

THE AUTHOR

Eva gained her MSc in developmental neuroscience from University College London and Yale University. In 2013, Eva received a MRC studentship and began her PhD at the Social, Genetic and Developmental Psychiatry Centre, King's College London, where she is working under the supervision of Professor Robert Plomin. She is interested in interdisciplinary research that brings together quantitative and molecular genetic approaches to investigate mechanisms that drive gene-environment correlation during development.
List of publications: https://scholar.google.co.uk/citations?user=MmKl9xsAAAAJ&hl=en
Twins Early Development Study: http://www.teds.ac.uk/

Eva Krapohl, King's College London

Biomarkers and Metabolomics

Fiona Hagenbeek, VU University Amsterdam

Data Collection NTR

Within ACTION, Fiona Hagenbeek is responsible for the new data collection in the Netherlands Twin Register (NTR) to aid in epigenetics and biomarker discovery for work package 3 and 5. The focus is on twin-children 7-12 years of age. The NTR data collection consists of four phases. In the first phase, the 'practical pilot', the new protocol for urine collection has been tested in 6 children. When this worked well, the second phase started. In the 'technical pilot', we collected first-morning urine and buccal-cell DNA in 10 twin pairs at two time points with an interval of approximately two weeks, to test the temporal stability of the selected assays and platforms. Currently, the third phase of collection is under way, in the 'biochemical study', we collect first-morning urine, buccal-cell DNA and parent-reported questionnaires in at least 100 twin-children selected for high aggression (CBCL Aggression Scale score > T65) and 100 controls.

Top row left: invitation and informed consent.
Top row right: Urine collection package.
Bottom row left: DNA collection package.
Bottom row right: portable freezer for transporting urine

Using the data collected in the 'biochemical study', the final selection of assays and platforms for the project will be determined. DNA isolated from buccal cells is used to confirm twin zygosity, for assessment of Single Nucleotide Polymorphisms (SNPs) and for epigenetic studies.

In the final wave of NTR data collection, the 'discovery phase', epigenetic profiles and biomarkers in urine will be collected in 200 monozygotic (MZ) twin pairs concordantly high on aggression, 200 MZ twin pairs concordantly low on aggression and 400 MZ twin pairs discordant on aggression.

This 'discovery phase' aims at establishing and validating potential biomarkers for aggression, which next will be validated in clinical samples.

Over the past 7 months we travelled throughout our country and collected urine and DNA from 116 families (see map for locations throughout the Netherlands).

THE AUTHOR

PhD student (Epigenetics, Biomarkers and Metabolomics) at the Vrije Universiteit Amsterdam, under the supervision of Prof. Dr. D.I. Boomsma, Prof. Dr. M. Bartels and Dr. H.H.M. Draisma.

During PhD, project responsible for new data collection in the ACTION consortia. Research activities will include investigating the interplay between genetics and metabolomics and searching for biomarkers for pediatric aggression.

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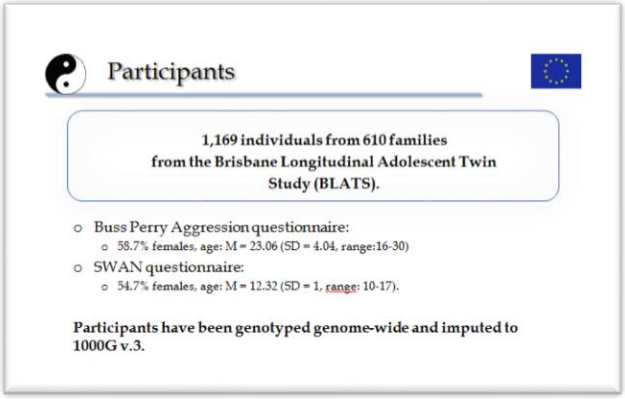
Fiona Hagenbeek, VU University Amsterdam

Predicting aggressive behavior from genetic risk for ADHD and ASD

Lucia Colodro Conde, QIMR Berghofer Medical Research Institute



Aggressive behavior is commonly comorbid with ADHD and autism and the three conditions are partially heritable. We investigated if the genetic risk for ADHD and autism could predict aggressive behavior. Information on aggressive behavior was collected with the Aggression Questionnaire (Buss & Perry, 1992) or with the items from the SWAN scale (Strengths and Weaknesses of ADHD-symptoms and Normal-behavior, Swanson et al., 2012) that overlap with the DSM criteria for oppositional defiant disorder. Data were available for 1,169 individuals from 610 families from the Brisbane Longitudinal Adolescent Twin Study, in Australia. Participants have been genotyped genome-wide and imputed to 1000G v.3. GWAS results from the latest Psychiatric Genomics Consortium meta analyses for ADHD and autism were used to calculate polygenic risk scores (PRS) for these disorders. Several PRS were calculated including SNPs associated with the trait at levels p <0.00000005, 0.00001, 0.001, 0.01, 0.05, 0.1, 0.5 or 1. We tested the associations of the PRS with aggressive behavior using linear mixed models in GCTA to account for the family structure.

At the behavioral level there are substantial associations between ADHD and aggression, but our results show no association between the genetic risk for ADHD or Autism and aggression. However, our results are limited by the number of participants as well as for power of the original PGC GWAS. Future research should explore the association between other psychiatry disorders with the development of aggression.

THE AUTHOR

Lucía Colodro Conde is a Post-doctoral researcher working at QIMR Berghofer Medical Research Institute in Brisbane, Australia.

Her background is in Clinical Psychology and Quantitative Genetics. She received her PhD in 2013 from the University of Murcia and was awarded a Post-doctoral Fellowship from the Seneca Foundation in Spain. Her interests are women's health, personality and psychopathology. She is working in the Action project under the supervision of Drs. Sarah Medland and Nick Martin.

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Lucía Colondro Conde,
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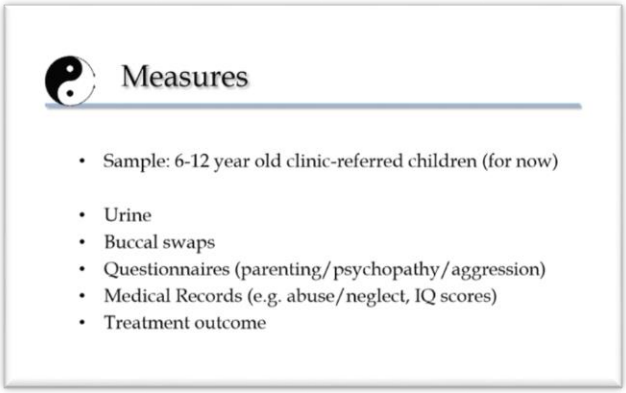
Clinical epidemiology (ACTION WP 2)

Peter J. Roetman, Curium-LUMC



The current works of Curium-LUMC are to (1) select clinical cases for the biomarker and epigenetic studies and (2) to make an inventarisation of the current clinical practice in prevention and treatment of aggression. Curium-LUMC is a Centre for Child and Adolescent Psychiatry.

The selection of clinical cases is made from clinic-referred children at Curium-LUMC. One of the main challenges is to implement the collection of samples and phenotypical data, while in the meantime guaranteeing the best possible care for the clients. Although obtaining approval from the medical ethical committee has proven lengthy, data collection will commence shortly.

Recently work has started on the second objective: the inventarisation of clinical practice. This objective is divided in two components. Firstly, the creation of an overview of guidelines of diagnostic, intervention and treatment of severe behavioral problems throughout Europe. Secondly, the collection of clinician's opinions on which components of diagnostics/intervention/prevention are deemed successful "core components". These expert opinions will be collected through semi-structured questionnaires.

Results of both projects will contribute to the creation, by work package 6, of a comprehensive framework on aggression and tools to improve clinical decision making processes combined with guidelines.

THE AUTHOR

Peter Roetman has been working as a PhD student at Curium-LUMC since April 2015.

His main task is to identify a group of clinical cases for whom a biobank infrastructure is established at Curium-LUMC, a Centre for Child and Adolescent Psychiatry, under the supervision of Prof. Robert Vermeiren and Dr. O. Collins.

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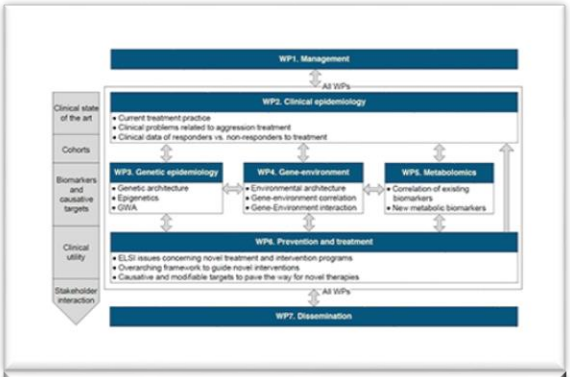
Peter J. Roetman,
Curium Leiden University Medical Center

Prevention and treatment (ACTION WP6)

Anne Hendriks, Vrije Universiteit Amsterdam



The role of WP6 within ACTION

Work package 6 aims at identifying (1) who is more likely to develop aggression and associated disorders, (2) who is likely to profit from prevention and intervention efforts, and (3) who is very unlikely to profit from prevention and intervention efforts. The first step towards this aim is to investigate and review literature on childhood aggression to gain more insight on which risk factors are associated with childhood aggression and which prevention and interventions programs have been used to reduce childhood aggression. The second step towards this aim is to combine and analyze the data that has been gathered by the partners to eventually create a comprehensive framework and tools to improve clinical decision making processes combined with guidelines. These tools will not only be for clinicians, but also for parents, teachers, general practitioners, etc. For this reason, it is important to also take the ethical, social, and legal implications into account, and whether these are different across countries in Europe.

Current and future works and tasks

1. Creating an overview of the data that has been collected by all the partners: which questionnaires have been used at which ages, and from which informants? There appears to only be a small overlap in questionnaires, so now the inspection takes place at item-level.
   The collection of an overview of all the data currently has two purposes: **(1) to assess** the phenotype aggression as measured in ACTION; **(2) to create** a harmonized dataset consisting of data from the NTR, CATSS, TCHAD, FinnTwin12, Qtwin, and Generation R.
2. Gathering literature on risk factors associated with childhood aggression, and interventions and preventions that have been implemented to improve childhood aggressive behaviors. Together with the data gathered by the partners, this theoretical background can be used to define a model for the clinical assessment of childhood aggression.

THE AUTHOR

Since August 2015, Anne has been working for ACTION as a PhD student at the Vrije Universiteit Amsterdam tutored by Prof. Catrin Finkenauer and Prof. Meike Bartels. Before this, she studied Child Development and Education at the University of Amsterdam.

Her main interests are child development and the implications of multiple informants.

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Anne Hendriks, VU University Amsterdam

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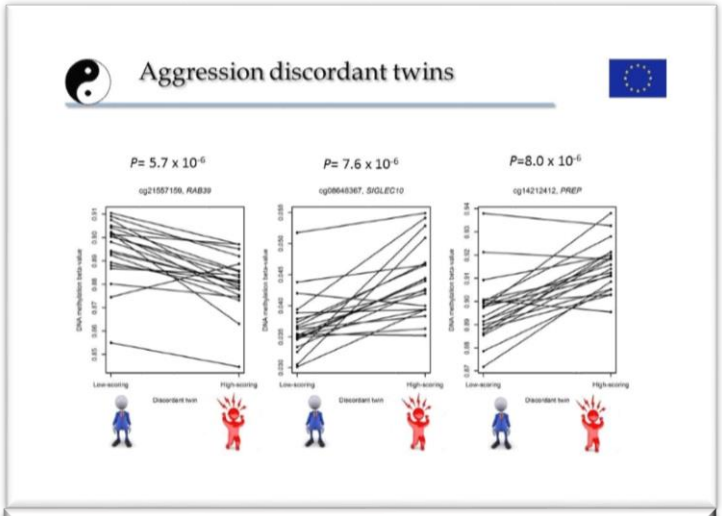
Epigenetics and aggression

Jenny van Dongen, Vrije Universiteit Amsterdam, Netherlands Twin Register

Epigenome-wide association study of aggressive behavior

During the Action meeting in Amsterdam I presented my recent work on epigenetics. I discussed the results of an epigenome-wide association study (EWAS) of aggressive behaviour based on an existing DNA methylation dataset (Illumina 450k array) in adult twins.

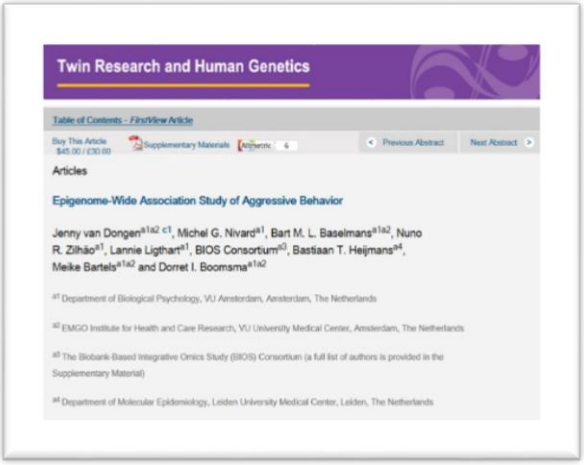


The slide shows the methylation levels in 20 aggression discordant adult monozygotic twin pairs at the top 3 CpG sites.

The association between aggression scored with the ASEBA Adult Self-Report (ASR) and DNA methylation in whole blood was tested in 2029 subjects (mean age=36.4 years).

The EWAS in the entire cohort revealed top sites at cg01792876 (chr8; 116,684,801, nearest gene=*TRPS1*, P=7.6 x 10^{-7}, FDR=0.18) and cg06092953 (chr18; 77,905,699, nearest gene= *PARD6G-AS1, P*=9.0 x 10^{-7}, FDR=0.18).

Because my dataset included monozygotic twins, I looked for twin pairs who were highly discordant for aggression. These pairs are rare, as aggression is a trait with substiantial heritability. Here, the top sites were cg21557159 (chr 11; 107,795,699, nearest gene= *RAB39,* P=5.7 x 10^{-6}, FDR=0.99), cg08648367 (chr 19; 51,925,472, nearest gene= *SIGLEC10,* P=7.6 x 10^{-6}, FDR=0.99), and cg14212412 (chr 6; 105,918,992, nearest gene= *PREP,* P= 8.0 x 10^{-6}, FDR=0.99).



The results of this EWAS are published in a special issue on Epigenetics of Twin Research and Human Genetics.

The article has already been published online, and is also available on our ACTION website.

http://journals.cambridge.org/displayAbstract

Funded by the European Commission under the Seventh Framework Programme

Replication and ongoing DNA sample collection.

As a next step, we are working with ACTION cohorts with data on aggression and DNA methylation, to replicate these results. In the ACTION project we will perform a new EWAS meta-analysis across cohorts.

To model the causal relations of DNA methylation and childhood aggression, DNA methylation will be measured in buccal swabs from young monozygotic twin pairs, who are concordant or discordant for aggression. These samples are currently being collected in the Netherlands Twin Register.

THE AUTHOR

Jenny works as a postdoc on the analysis of DNA methylation data in combination with phenotypic and genetic data from twins and families.

She is part of ACTION work package 3.

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Jenny van Dongen, VU University Amsterdam

11th Workshop on Neonatology

The "11th International Workshop on Neonatology – From the womb to the adult", took place in Cagliari, Italy (October 26-31), following the success of the previous editions which saw the overall presence of more than 5700 participants. Workshop 11 had **more than 1000 partecipants** and was accompanied by many national and international institutions and scientific societies (**Italian Ministry of Health** and **UNICEF**, just to name a few).

The title of the workshop "From the womb to the adult" underscores this itinerary: a view towards the future of perinatology through debate and the contribution of the Workshop, but above all the trajectory that assistance, research and teaching will follow in the coming years, in an integrated way with the medicine of the adult. Some of the world's most renowned specialists from forty different nations have been present in Cagliari in these years. They have created a network of scientific and human relations that have changed, improved and enriched the scientific community. This year the proceedings of the Workshop have been be published in the "Journal of Pediatric and Neonatal Individualized Medicine" (JPNIM). In past years the journals that took care of the proceedings were "Medical and Surgical Pediatrics", "Journal of Chemotherapy", "Journal of Maternal Fetal Neonatal Medicine", "Clinica Chimica Acta" and JPNIM. Over the years, the Workshop has made its impression on science and has promoted a lively discussion in an always serene and extremely constructive atmosphere.

This year's meeting has been organized with the patronage of the Italian Society of Neonatology (SIN), the Italian Society of Pediatrics (SIP), the Italian Society of Perinatal Medicine (SIMP), the Italian Federation of Pediatricians (FIMP), the Union of European Neonatal and Perinatal Societies (UENPS), the Union of Mediterranean Neonatal Societies (UMENS), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and lastly by the Italian National Observatory of Residents in Paediatrics (ONSP).

The meeting was dedicated to neonatologists, pediatricians, obstetricians, perinatologists, laboratory researchers, pediatric cardiologists and all those who work with the newborn.

Besides the traditional appointment of the International Workshop, there were seven satellite meetings: "A SPECIAL day" for young fellows; the tenth edition of the "Terra di Sardegna Meeting on Neonatological Nursing" with the participation of nurses from more than ten neonatal intensive care units; the second meeting titled "New Frontiers in Preventive and Social Pediatrics" that focused attention on common neonatal problems and immunity; the 1st perinatal meeting "The future of Perinatal Medicine" devoted to clinical and organizative topics; the 5th International Conference on Neonatal and Pediatric Laboratory Medicine during which the European Project ACTION has been presented; finally, the 2nd International Course on Perinatal Pathology, where perinatologists and pathologists came together to address systematically the cutting-edge topic of stem cells and their practical consequences in neonatology.

Session IV. The Action project: Dissemination

The University of Cagliari presented the ACTION'goals on Friday 30, during the afternoon session (5 pm - 8 pm).

- **Chairman**:
  Prof. Giovanni Mauro Carta (Cagliari, Italy)

- **Moderators**:
  Prof. Luigi Atzori (Cagliari, Italy),

Talks:

- **The ACTION project**
  Prof. Dorret Boomsma (Amsterdam, The Netherlands)
- **A website for parents and researchers**
  Matteo Mauri (Cagliari, Italy)
- **Biomarkers of aggression: metabolomics**
  Antonio Noto (Cagliari, Italy)
- **Brain mysteries from the womb to the adult: is aggression congenital?**
  Prof. Vassilios Fanos (Cagliari, Italy)
- **Practical impact of ACTION project**
  Prof. Giuseppe Buonocore (Siena, Italy)

- **Discussants**:
  Prof. Pierluigi Caboni (Cagliari, Italy)
  Prof. Andrea Rinaldi (Cagliari, Italy)
  Dr. Michele Mussap (Genoa, Italy)
  Dr. Antonio Del Vecchio (Bari, Italy)

Prof. Boomsma presents ACTION
Full photogallery available in the ACTION website

Interview with Prof. Fanos (Italian TV Videolina)

ACTION Publications

The ACTION Project is proud to present the first series of publications, written during this first year of activities.

Some details: 34 authors, 6 Journals, 9 papers (2 Workshop proceedings, 3 Journal articles, 2 PubMed reviewed Journal articles, 2 abstracts).

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http://www.action-euproject.eu/biblio

- D. I. Boomsma, **"Aggression in children: unravelling the interplay of genes and environment through (epi)genetics and metabolomics"**, *Journal of Pediatric and Neonatal Individualized Medicine (JPNIM)*, vol. 4, no. 2, pp. 97-102, 2015.
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  DOI, Google Scholar, BibTex

- A. Noto and Fanos V., "**Selected Abstracts of the 11th International Workshop on Neonatology. ABS 84, Biomarkers of aggression: metabolomics**", *Journal of Pediatric and Neonatal Individualized Medicine (JPNIM)*, vol. 4, no. 2, pp. 210-211, 2015.
  DOI, Google Scholar, BibTex

- M. Manchia and Fanos V., "**Selected Abstracts of the 11th International Workshop on Neonatology. ABS 100, Brain mysteries from the womb to the adult: is aggression congenital?**", *Journal of Pediatric and Neonatal Individualized Medicine (JPNIM)*, vol. 4, no. 2, p. 231, 2015.
  DOI, Google Scholar, BibTex

Contributors