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

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Biomarkers and Metabolomics of Childhood Aggression

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Introduction

- Research in the biochemical basis of aggressive disorders has focused on putative biomarkers of various classes in isolation:
  - Aggression biochemistry research will benefit from a more holistic approach as provided by metabolomics (Hagenbeek, et al. 2016).
  - A previous metabolomics study suggested at a relationship between serum amino acid levels with aggression and psychopathy in adult males (Gulsun, et al. 2016).
- By identifying urinary biomarkers of aggression **ACTION** aims to unravel processes and pathways leading to childhood aggression.

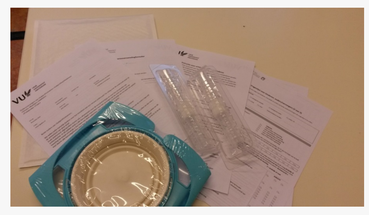
Table 1. List of candidate biomarkers for ACTION

Substance P	Cholecystokinin	Total oxidative capacity
Enkephalin- Methionine	Serum amyloid albumin	8-hydroxydeoxyguanosine
Enkephalin-Leucine	Interleukin-6	Ox-bilirubin (biopyrin)
Beta-endorphin	Procalcitonin	Hexanoyl lysine adduct
Dynorphin	Albumin	Malondialdehyde
Neurotensin	Creatinine	Neopterin
C-peptide	Glucose	Cotinine

Data collection & Pilot Studies

- **ACTION** aims to simultaneously collect **DNA** material, **urine** samples, **health** information & **aggression** score in children of **7 to 12** years of age registered with the Netherlands Twin Register (**NTR**).
- Prior to start data collection a **Practical Pilot** was conducted to test the urine collection protocol in 6 non-aggressive children.

Invitation & informed consents



Urine collection material & questionnaires

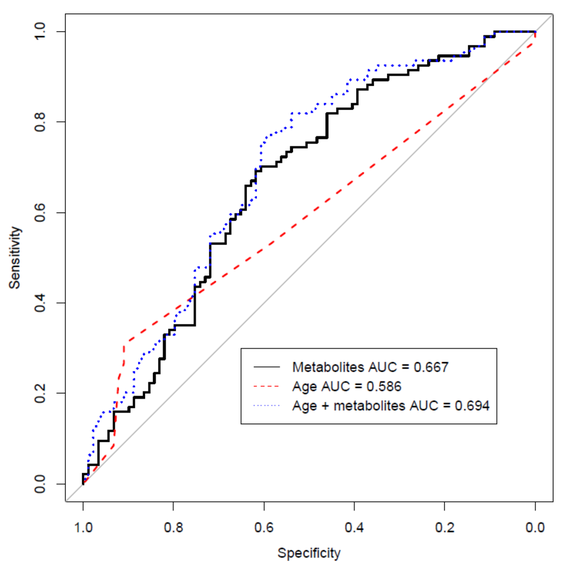
DNA collection kit

- In the **Technical Pilot** the temporal stability of our selected biomarkers (see **Table 1**), the **GC-MS biogenic amines** platform and the **UPLC-MS/MS organic acids** platform was evaluated in 20 non-aggressive twin children (60% males).
  - Temporal stability was assessed by comparing urine samples from two separate days (approximately 19 days apart).
  - Of the 19 selected biomarkers, 5 are discontinued, the remaining 14 biomarkers and 2 additional biomarkers will be continued in the next phase.
  - Both metabolomics platforms will be continued in the next phase, as well as an additional metabolomics platform targeting steroids.

Biochemical Study

- **Aim:** Attempt to detect contrast between aggression cases and controls using a broad metabolomics approach.
- **Participants**: 222 twin children (50% females) of 6-12 years of age selected for high- or low-aggression status (89 cases & 133 controls).
  - After cleaning, metabolomics data and current aggression status was available for 100 cases and 90 controls.
- **Lasso regression**: lasso regression was unable to find significantly predictive metabolites for the amines platform. Lasso regression for the organic acids resulted in a model with 4 metabolites:2-hydroxybutyric acid, Succinic acid, Aspartic acid & Uracil.
  - The predictive value of the model including the 4 organic acids is relatively poor (see **Fig 1**), though it outperforms age only and adding age to the model increases the predictive accuracy.
- **Univariate Follow-up:** GEE models correcting for family structure and including age, vitamin use and batch effect as covariates found a significant association with aggression for 2-hydroxybutyric acid ($\beta = 0.31$, $p = 0.04$).
- **Further Analyses:** The lasso model selection analyses will be repeated in a mixed-model framework in order to correct for family structure.

Fig. 1: Predictive accuracy lasso regression metabolites organic acids platform.



Discovery Phase

- The aim of the **Discovery Phase** is the establishment of putative childhood aggression biomarkers as identified by the **Biochemical Study** in 600 monozygotic (**MZ**) twin pairs selected for their aggression (dis-) concordance status.

Fig. 2: Participation rate of invited twin pairs per concordance status

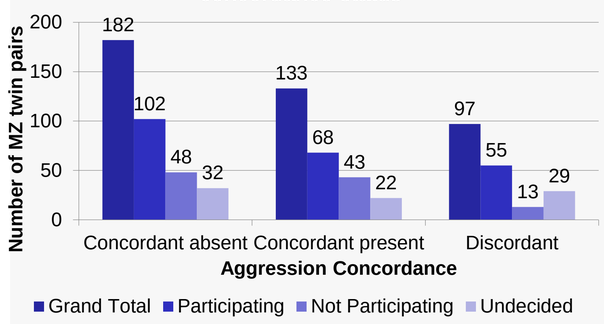
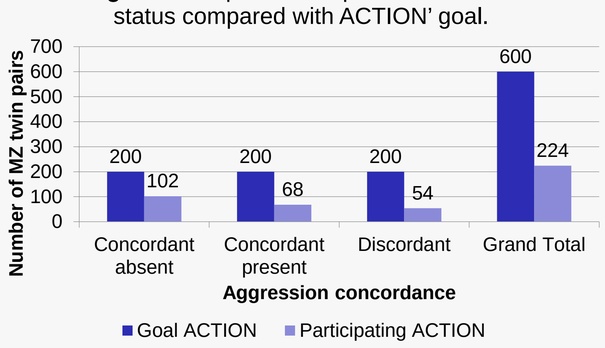


Fig. 3: Participation rate per concordance status compared with ACTION' goal.



Conclusions & Future Directives

- Metabolites can be reliably and reproducibly measured in urine samples of 7-12 year old children as shown in the **Technical Pilot**.
- Lasso regression for the organic acids identified a model including 4 metabolites as best predicting aggression case/control status in children in the **Biochemical Study**.
- The biomarkers and the steroid metabolomics platform of the **Biochemical Study** are currently being analyzed, statistical analyses will follow a.s.a.p.
- Recruitment and sample collection for the **Discovery Phase** will continue, with an anticipated end in the summer of 2017.
- After discovery of putative biomarkers in the **Discovery Phase**, these findings will be validated in a clinical cohort.

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