



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

## Heritability of cortisol production and metabolism throughout adolescence: a twin study

**Citation for published version:**

van Keulen, BJ, Dolan, CV, Andrew, R, Walker, B, Hulshoff Pol, HE, Boomsma, DI, Rotteveel, J & finken, MJJ 2019, 'Heritability of cortisol production and metabolism throughout adolescence: a twin study', *The Journal of Clinical Endocrinology & Metabolism (JCEM)*. <https://doi.org/10.1210/clinem/dgz016>

**Digital Object Identifier (DOI):**

[10.1210/clinem/dgz016](https://doi.org/10.1210/clinem/dgz016)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher's PDF, also known as Version of record

**Published In:**

The Journal of Clinical Endocrinology & Metabolism (JCEM)

**General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



## Heritability of cortisol production and metabolism throughout adolescence: a twin study

*Britt J. van Keulen<sup>1</sup>, Conor V. Dolan<sup>2</sup>, Ruth Andrew<sup>3</sup>, Brian R. Walker<sup>3,4</sup>, Hilleke E. Hulshoff Pol<sup>5</sup>, Dorret I. Boomsma<sup>2</sup>, Joost Rotteveel<sup>1</sup>, Martijn J.J. Finken<sup>1</sup>*

<sup>1</sup> Emma Children's Hospital, Amsterdam UMC, Vrije Universiteit Amsterdam, Pediatric Endocrinology, Amsterdam, The Netherlands

<sup>2</sup> Department of Biological Psychology, Vrije Universiteit Amsterdam, The Netherlands

<sup>3</sup> Centre for Cardiovascular Science, University of Edinburgh, Queen's Medical Research Institute, Edinburgh, United Kingdom

<sup>4</sup> Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

<sup>5</sup> Department of Psychiatry, Brian Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands

**Grants:** BRW is a Wellcome Trust Investigator. RA and BRW were supported by a British Heart Foundation Programme Grant, and by a Wellcome Trust equipment grant. This work was supported by the Netherlands Organization for Scientific Research (NWO, 51.02.060, 668.772 ; NWO-MagW 480-04-004; NWO/SPI 56-464-14192). DIB acknowledges KNAW Academy Professor Award (PAH/6635).

**Disclosure summary:** not applicable

### Key words

Cortisol production

Cortisol metabolism

Classical Twin design

Adolescence

Heritability

### Corresponding author (including reprint requests)

Britt J van Keulen, MD

Emma Children's Hospital

Amsterdam UMC

Vrije Universiteit Amsterdam

Pediatric endocrinology

De Boelelaan 1117

1081 HV Amsterdam, The Netherlands

[b.j.vankeulen@amsterdamumc.nl](mailto:b.j.vankeulen@amsterdamumc.nl)

tel +31-20-4444444

© Endocrine Society 2019. jc.2019-00993. See [endocrine.org/publications](http://endocrine.org/publications) for Accepted Manuscript disclaimer and additional information.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

## Abstract

Context: Inter-individual differences in cortisol production and metabolism emerge with age, and may be explained by genetic factors.

Objective: To estimate the relative contributions of genetic and environmental factors to inter-individual differences in cortisol production and metabolism throughout adolescence.

Design: Prospective follow-up study of twins.

Setting: Nationwide register.

Participants: 218 mono- and dizygotic twins (N=109 pairs) born between 1995-1996, recruited from the Netherlands Twin Register. Cortisol metabolites were determined in 213, 169 and 160 urine samples at the ages of 9, 12 and 17y, respectively.

Main outcome measures: The total contribution of genetic factors (broad-sense heritability), and shared and unshared environmental influences to inter-individual differences in cortisol production, and activities of 5 $\alpha$ -reductase, 5 $\beta$ -reductase, 11 $\beta$ -hydroxysteroid dehydrogenases and cytochrome P450 3A4.

Results: For cortisol production rate at the ages of 9, 12 and 17y, broad-sense heritability was estimated as: 42%, 30% and 0%, respectively and the remainder of the variance was explained by unshared environmental factors. For cortisol metabolism indices, the following heritability was observed: for the A-ring reductases (5 $\alpha$ - and 5 $\beta$ -reductases), broad-sense heritability increased with age (to >50%), while for the other indices (renal 11 $\beta$ -HSD2, global 11 $\beta$ -HSD and CYP3A4), the contribution of genetic factors was highest (68%, 18% and 67%, respectively) at age 12y.

Conclusions: The contribution of genetic factors to inter-individual differences in cortisol production decreased between 12 and 17y, indicative of a predominant role of individual circumstances. For

cortisol metabolism, distinct patterns of genetic and environmental influences were observed, with heritability that either increased with age or peaked at age 12y.

**Précis**

In a longitudinal twin study, we found that the contribution genetic factors to cortisol production decreased over adolescence, while it increased or peaked at age 12y for cortisol metabolism.

Accepted Manuscript

## Introduction

Cortisol, the main product of the hypothalamus-pituitary-adrenal (HPA) axis, is a crucial steroid hormone in the physiological stress response following homeostasis disturbance (1). Dysregulation of HPA axis activity has been associated with cardiovascular diseases and psychiatric conditions, e.g., major depressive disorder, post-traumatic stress disorder, panic disorder, and chronic anxiety (2-4). It has been recognized that experiences in early life may induce permanent alterations in the settings of several endocrine systems, including the HPA axis (5,6).

There is a paucity of data on the magnitude of the contribution of genetic factors to variance in HPA axis activity. Several studies in monozygotic (MZ) and dizygotic (DZ) twins addressed the relative contributions of genetic and environmental factors on serum and salivary cortisol levels. However, the magnitude of the heritability estimates varies (Table 1). A meta-analysis of five twin studies published before 2001, of which 4 were conducted in adults and 1 in both children and adults, estimated the heritability of basal cortisol assessed in serum or saliva at 62% (7). The total sample size in the meta-analysis was small (209 MZ and 190 DZ pairs), and power analyses included in the paper indicated that the statistical power to distinguish between genetic and shared environmental influences was low. Later studies reported lower heritability estimates for salivary cortisol levels, and showed that those estimates differed at different time points of the circadian cortisol rhythm (8-11). In adults, the heritability of cortisol level was 32-34% in samples obtained directly after awakening or 30 minutes post-awakening (9). In the evening samples obtained from these adults, the heritability was found to equal zero. In children, the heritability estimates were 28%, 60%, and 8%, directly after awakening, 30 minutes post-awakening, and in the evenings, respectively (8). In summary, previous twin research consistently found that variation in morning salivary, serum and hair cortisol levels appears to be at least partially heritable (Table 1). Cortisol secretion varies with age, and inter-individual differences in diurnal cortisol levels are presumed to emerge during the second decade of life (12).

Earlier studies have focused on cortisol levels in serum or salivary. These cortisol levels represent the net effect of cortisol production and metabolism. The contributions of genetic and environmental factors to individual differences in cortisol production or metabolism, as determined by cortisol metabolite excretion in urine, have yet to be studied. Day-to-day excretion of cortisol in the urine is moderately stable (13). Cortisol is metabolized to cortisone by 11 $\beta$ -hydroxysteroid dehydrogenase (HSD) type 2 in the kidney, while the reverse reaction occurs by 11 $\beta$ -HSD type 1 in liver and adipose tissue. Cortisol is also metabolized irreversibly by the A-ring reductases (5 $\alpha$ -reductase and 5 $\beta$ -reductase), and cytochrome P450 (CYP) 3A4 in the liver. Cortisol metabolism is stable across the menstrual cycle (14). The aim of the current study was to focus on indices of cortisol production and metabolism across adolescence, and to estimate the relative contributions of genetic and environmental factors to cortisol production and metabolism in a sample of children who were registered at birth in the Netherlands Twin Register (NTR). The twins took part in a longitudinal study and were seen at 9, 12 and 17 years of age (15).

## **Methods**

### *Participants*

We conducted a prospective follow-up study in mono- and di-zygotic twin pairs. Participants in this study were recruited from the Netherlands Twin Register (NTR) (16,17), and invited to take part in the BrainScale study of cognition, hormones and brain development (15,18). Parents of twins born between 1995 and 1996 were invited by letter four to eight weeks before the ninth birthday of the twins. Two weeks later, the parents were approached by phone, to explain about the study and to ask them if they consented to their children taking part. Of the 214 families who were approached, 109 consented to take part (51%). Seventy-eight and seventy-three percent of the participants took part in the follow-up study, and provided samples at the ages of 12 and 17 years, respectively (Figure 1).

Information on demographics, gestational age, birth weight and other pre- and perinatal variables was collected by questionnaire at the time of NTR registration (19). Twins were categorized based on zygosity and sex: MZM (monozygotic males), DZM (dizygotic males), MZF (monozygotic females), DZF (dizygotic females), DZOS (dizygotic opposite sex male-female or female-male). To establish zygosity, participants were requested to collect buccal swabs from which DNA was isolated. All DNA samples were tested for genome-wide single nucleotide polymorphic (SNP) markers (20).

BrainScale is a collaborative project between the Netherland Twin Register at the Vrije Universiteit Amsterdam and University Medical Center Utrecht. The project was approved by the Central Committee on Research Involving Human Subjects of The Netherlands (CCMO), and studies were performed in accordance with the Declaration of Helsinki. Parents signed informed consent forms for the children and for themselves. Children signed their own informed consent forms at the third measurement. Parents were compensated for travel expenses, and children received a present or gift voucher at the end of the testing day. In addition, a summary of cognition scores and a printed image of their T1 brain MRI scan, when available, were provided afterwards

The current study was approved by the medical ethics committee of the Amsterdam UMC, location VUmc.

#### *Study protocol*

At the ages of 9, 12 and 17 years, participants visited the study site for different tests. In the week prior to the study visit, urine samples were collected upon awakening in specially provided tubes. Participants or their parents were requested to store the tubes in their refrigerator, and to bring them to the study visit. Samples were subsequently stored at -20 and -80 degrees Celsius, and thawed only once just before analysis. Urine samples were available from 47 monozygotic (23 male, 24 female) and 62 dizygotic twin pairs (22 male, 21 female, 19 opposite sex).

Participants were instructed to bring any packages of recently used medication to the study visits, revealing that there was hardly any recent use of medication in our sample.

#### *Laboratory analysis*

Analysis of cortisol metabolites was conducted at the Edinburgh Clinical Research Facility Mass Spectrometry Core Laboratory. Glucocorticoid metabolites were measured by gas chromatography-tandem mass spectrometry (GC-MS/MS) (21). Samples were analyzed in fifteen batches. Ratios of cortisol metabolites representing the activities of various enzymes involved in cortisol metabolism were calculated, as depicted in Table 2.

#### *Statistical analysis*

Outliers, defined as any value greater than 3 standard deviations above the phenotypic mean, were excluded from statistical analysis (on average six per index). Twin pairs with highly discordant outcomes, as assessed by visual inspection of scatterplots, were also removed (on average 0.76 per index), given their impact on the MZ and DZ correlations. We corrected for batch effects by fitting a random effects model to the twin data, which included batch as a random effect (22). As the sampling unit is twin pairs, we included in the model family as a random effect to estimate the MZ and DZ twin (intra-class) correlations. These analyses were done in R 3.4.2. using the nlme library (Nonlinear Mixed-Effects Models) (23,24). The batch-corrected phenotypic data were subject to subsequent analyses, as described below.

The classical twin design exploits the fact that MZ twins share 100% of their alleles identically by descent (IBD; from the biological parents), and DZ twins on average share 50% of their alleles IBD (25). This difference in genetic relatedness allows us to estimate the relative contributions of genetic and environmental factors to phenotypic individual differences in terms of genetic and environmental variance components. In practice, either an ACE model or an ADE model is fitted to the twin data, where A stands for additive genetic effects, D for non-additive or dominance (genetic)



effects, C for shared (or common) environmental effects, and E for unshared environmental effects. MZ and DZ pairs differ in their genetic relatedness, but share the pre- and postnatal environment. The extent to which resemblance in both types of twins is not explained by differential genetic resemblance, is the basis for identification of common environmental factors. An ACE model is fitted to twin data if the MZ phenotypic correlation ( $r_{MZ}$ ) is smaller than two times the DZ correlation ( $r_{DZ}$ ;  $r_{MZ} < 2 * r_{DZ}$ ). With 2 groups of relatives, i.e. MZ and DZ twins, it is not possible to estimate four variance components, i.e. a model including non-additive genetic factors in addition to common environment is not identified. A choice needs to be made to evaluate an ACE or ADE model. If ( $r_{MZ} > 2 * r_{DZ}$ ) an ADE model is chosen. Unshared environmental factors incorporates aspects of the environment that are child specific and results in differences between twin pairs, i.e. the differences within MZ pairs are due to these factors. Unshared environmental factors also includes measurement error. Path diagrams of the ACE and ADE models are shown in Figure 2. We fitted a series of univariate models.

We used OpenMx to fit the ACE or ADE model to the batch corrected twin data (26). The genetic and environmental variance components were estimated by maximum likelihood. We included as fixed covariates in the model sex and gestational age, since these factors have previously been associated with HPA axis activity (12,27). Below, we present the estimates of total standardized variance components. The standardized variance components were obtained by dividing by the total variance.

## Results

A total of 218 twins who were born in the Netherlands were enrolled in this study, including 94 monozygotic and 124 dizygotic twins. The monozygotic pairs included 23 MZM twin pairs and 24 MZF twin pairs. The dizygotic twins included 22 DZM twin pairs, 21 DZF twin pairs, and 19 DOS twin pairs. In total, 213, 167 and 162 samples were analyzed at 9, 12 and 17 years of age, respectively.

Fifty percent ( $n=109$ ) of all participants was male. Mean [ $\pm$ SD] gestational age was 36.8 weeks [ $\pm 1.72$ ] and mean [ $\pm$ SD] birthweight was 2,602 gram [ $\pm 475$ ]. Participants were tested at 9.1 [ $\pm 0.1$ ], 12.2

[ $\pm 0.3$ ] and 17.2 [ $\pm 0.2$ ] years of age. Mean Standard Deviation Score (Z Score) [ $\pm$ SD] body mass index (weight(kg)/height(m)<sup>2</sup>) was 0.14 [ $\pm 0.93$ ], 0.45 [ $\pm 1.00$ ] and 0.26 [ $\pm 1.57$ ] at the ages 9, 12 and 17 years, respectively (28).

The twin correlations and variances for each index are presented in Table 3. The DZ correlations (average .257) were lower than the MZ correlations (average .415) in 17 of the 21 phenotypes, pointing towards a relatively simple additive (AE) genetic model. Thirteen of the phenotypes appeared to be consistent with an ACE model. We note that some correlations were zero (MZ: 5 $\beta$ -reductase activity at age 12; DZ: Renal 11 $\beta$ -HSD type 2 activity). We attribute this to sampling fluctuation, given the relatively small sample sizes.

The relative contributions of genetic (additive and non-additive), shared environmental, and unshared environmental factors, along with 95% confidence intervals, are displayed in Table 4. At age 9y, the contribution of variation in the cortisol production rate was explained for approximately forty percent by genetic factors and sixty percent by unshared environmental factors. The contribution of genetic factors to the inter-individual differences in cortisol production rate decreased with age. At age 17y, genetic factors were no longer contributing to the cortisol production rate. Variation at this age is mainly (for 79%) explained by unshared environmental factors. For indexes representing A-ring reductases activity, the contribution of genetic factors to inter-individual differences was found to increase with age. The contribution of genetic factors to the inter-individual differences in activities of renal 11 $\beta$ -HSD type 2 activity, cytochrome P450 3A4 and 11 $\beta$ -HSD first increased from 9 to 12 years of age, and then decreased from 12 to 17 years.

## Discussion

In this longitudinal study of twin pairs followed between the ages of 9 and 17 years, we demonstrated the relative contributions of genetic and environmental factors to the indices of cortisol production and metabolism throughout adolescence. The most important finding from our study is that the environment plays a key role in the production of cortisol, evidenced by the

predominant and increasing contribution with age of unshared environmental factors. In addition, we found distinct patterns of genetic and environmental contribution to the different cortisol-metabolizing pathways.

A previous meta-analysis of twin studies estimated heritability of basal cortisol, representing the net effect of cortisol production and elimination, at 62% (7). Despite this observation, others demonstrated no significant SNP heritability for plasma or salivary morning cortisol (29). The interpretation of such discrepant findings is complicated, when considering that approximately fifty percent of the variance in salivary cortisol was dependent on day-to-day fluctuations (30).

In our study, cortisol production was mainly determined by unshared environmental factors already at age 9y, and the contribution of unshared environmental factors was found to increase with age. This lends support to previous observations suggesting that the settings of the HPA axis are mainly determined by individual circumstances (31). There is overwhelming evidence from animal experiments and epidemiological studies demonstrating that experiences in early life may program future HPA axis activity, with data linking poorer quality of parental care to increased HPA axis activity along with increases in mental illnesses and cardiovascular diseases (32-34). These observations could be attributed to increased DNA methylations status and a reduced expression of the glucocorticoid receptor (GR) promotor in hippocampal regions (6,35,36). However, more recent evidence suggests that the time window in which epigenetic programming could occur, may extend into adulthood (37-39). In male middle-aged twins, saliva cortisol levels showed significant cortisol heritability estimates for laboratory measures, but not for measures in the home situation, suggesting that genetic factors influence cortisol responses to specific environmental stressors (40).

In animal experiments, the influences of early life experiences can be observed by randomly allocating offspring into groups with different amounts of exposure to the factor of interest, whereas in humans determining the long-term impact of life experiences on HPA axis activity is more challenging. Previous research in humans linked early-life experiences like battering, neglect,

emotional maltreatment, perinatal malnutrition, low birthweight and prematurity with future HPA axis activity (33,41-44). The findings obtained from these studies are inevitably confounded by factors associated with both exposure and outcome, such as low socio-economic class and low household income. Twin studies offer a powerful tool to study environmental contributions, by controlling for the family background.

In our study, cortisol metabolism was, in contrast to cortisol production, considerably influenced by genetic constitution, and our findings suggest distinct patterns of genetic and environmental contribution to the different metabolic pathways. The activities of the A-ring reductases are, unlike cortisol production, less influenced by unshared environmental factors, especially later in life. Variation in A-ring reductases activity was for 0-23%, 0-51% and 51-66% explained by (non-additive and additive) genetic factors at 9, 12 and 17 years of age, respectively, indicating a predominant role of genetic constitution in the regulation of A-ring reductases with age. The influences of genetic constitution on the activities of 11 $\beta$ -HSD isozymes and CYP3A4 increased from 9 to 12 years, and then decreased from 12 to 17 years of age. An explanation for the varying influences found for these enzymes may lie in a complex interplay between cortisol metabolizing enzymes and the hormonal regulators of puberty and the pubertal growth spurt, in particular growth hormone (GH) and insulin-like growth factor 1 (IGF-1). A previous twin study has shown that the pubertal growth spurt, peaking between 12 and 14 years, is strictly genetically regulated (45). In addition, there is strong evidence suggesting that these hormones affect the clearance of cortisol (14,46,47).

Our study has several strengths and limitations. The major strength of our study was the long-term follow-up. Furthermore, participants were recruited from a nationwide twin registry and the numbers lost to follow-up were acceptably low. Consequently, selection bias is unlikely to explain our results. Another strength of our study was the use of GC/MS/MS analysis, providing highly reliable measurements. Thus, the findings as presented are unlikely to be explained by measurement error. However, our study also has its limitations. Participants were requested to collect early-morning

urine samples. Preferably, a 24h urine sample would have been analyzed, since cortisol is secreted in a circadian rhythm. Next, sampling started no earlier than at age 9y, so that there remains a lack of knowledge on the genetic and environmental etiology in early childhood. Moreover, differences between boys and girls were observed, necessitating further research. Additionally, due to limited sample size, resulting in large confidence intervals and some remarkable twin correlations (e.g. MZ correlation < DZ correlation for cortisol production rate at age 17y), our results have to be interpreted cautiously. Finally, the collected samples were not randomly distributed across the different analytical batches, which may have led to systematic error. More specifically, samples from twin pairs were allocated in the same batches, which might overestimate the contribution of shared environmental factors. Therefore, random correction for batch effect was carried out.

Accepted Manuscript

## Conclusion

Our current findings, along with previous observations, emphasize the significant role of individual circumstances on the settings of the HPA axis. Notably, the contribution of unshared environmental factors on cortisol production was considerable and was found to increase with age, implicating a predominant role of individual circumstances with ageing. In contrast to cortisol production, cortisol metabolism was considerably influenced by genetic constitution, and heritability of A-ring reductases was found to increase with age, resulting in a peak of the genetic contribution at the age of 17 years. For 11 $\beta$ -HSD isozymes and cytochrome P450 3A4, this peak was found at the age of 12 years.

## Acknowledgements

We thank the twins and their parents for making this study possible. We thank the staff of the Edinburgh Mass Spectrometry Core for specialist support. BRW is a Wellcome Trust Investigator. RA and BRW were supported by a British Heart Foundation Programme Grant, and by a Wellcome Trust equipment grant. This work was supported by the Netherlands Organization for Scientific Research (NWO, 51.02.060, 668.772 ; NWO-MagW 480-04-004; NWO/SPI 56-464-14192). DIB acknowledges KNAW Academy Professor Award (PAH/6635).

## References

1. Liyanarachchi K, Ross R, Debono M. Human studies on hypothalamo-pituitary-adrenal (HPA) axis. *Best Pract Res Clin Endocrinol Metab.* 2017;31(5):459-473.
2. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiol Rev.* 2014;94(4):1027-1076.
3. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci.* 2006;8(4):383-395.
4. Goodyer IM, Park RJ, Herbert J. Psychosocial and endocrine features of chronic first-episode major depression in 8-16 year olds. *Biol Psychiatry.* 2001;50(5):351-357.
5. Barker DJ. Fetal origins of coronary heart disease. *Br Heart J.* 1993;69(3):195-196.
6. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci.* 2004;7(8):847-854.
7. Bartels M, Van den Berg M, Sluyter F, Boomsma DI, de Geus EJ. Heritability of cortisol levels: review and simultaneous analysis of twin studies. *Psychoneuroendocrinology.* 2003;28(2):121-137.
8. Gustafsson PA, Gustafsson PE, Anckarsater H, Lichtenstein P, Ljung T, Nelson N, Larsson H. Heritability of cortisol regulation in children. *Twin Res Hum Genet.* 2011;14(6):553-561.
9. Kupper N, de Geus EJ, van den Berg M, Kirschbaum C, Boomsma DI, Willemsen G. Familial influences on basal salivary cortisol in an adult population. *Psychoneuroendocrinology.* 2005;30(9):857-868.
10. Bartels M, de Geus EJ, Kirschbaum C, Sluyter F, Boomsma DI. Heritability of daytime cortisol levels in children. *Behav Genet.* 2003;33(4):421-433.
11. Ouellet-Morin I, Brendgen M, Girard A, Lupien SJ, Dionne G, Vitaro F, Boivin M. Evidence of a unique and common genetic etiology between the CAR and the remaining part of the diurnal cycle: A study of 14 year-old twins. *Psychoneuroendocrinology.* 2016;66:91-100.
12. Wudy SA, Hartmann MF, Remer T. Sexual dimorphism in cortisol secretion starts after age 10 in healthy children: urinary cortisol metabolite excretion rates during growth. *Am J Physiol Endocrinol Metab.* 2007;293(4):E970-976.
13. Rosmalen JG, Kema IP, Wust S, van der Ley C, Visser ST, Snieder H, Bakker SJ. 24 h urinary free cortisol in large-scale epidemiological studies: short-term and long-term stability and sources of variability. *Psychoneuroendocrinology.* 2014;47:10-16.
14. Finken MJ, Andrews RC, Andrew R, Walker BR. Cortisol metabolism in healthy young adults: sexual dimorphism in activities of A-ring reductases, but not 11beta-hydroxysteroid dehydrogenases. *J Clin Endocrinol Metab.* 1999;84(9):3316-3321.
15. van Soelen IL, Brouwer RM, Peper JS, van Leeuwen M, Koenis MM, van Beijsterveldt TC, Swagerman SC, Kahn RS, Hulshoff Pol HE, Boomsma DI. Brain SCALE: brain structure and cognition: an adolescent longitudinal twin study into the genetic etiology of individual differences. *Twin Res Hum Genet.* 2012;15(3):453-467.
16. Boomsma DI, de Geus EJ, Vink JM, Stubbe JH, Distel MA, Hottenga JJ, Posthuma D, van Beijsterveldt TC, Hudziak JJ, Bartels M, Willemsen G. Netherlands Twin Register: from twins to twin families. *Twin Res Hum Genet.* 2006;9(6):849-857.
17. Boomsma DI, Vink JM, van Beijsterveldt TC, de Geus EJ, Beem AL, Mulder EJ, Derks EM, Riese H, Willemsen GA, Bartels M, van den Berg M, Kupper NH, Polderman TJ, Posthuma D, Rietveld MJ, Stubbe JH, Knol LI, Stroet T, van Baal GC. Netherlands Twin Register: a focus on longitudinal research. *Twin Res.* 2002;5(5):401-406.
18. Teeuw J, Brouwer RM, Koenis MMG, Swagerman SC, Boomsma DI, Hulshoff Pol HE. Genetic Influences on the Development of Cerebral Cortical Thickness During Childhood and Adolescence in a Dutch Longitudinal Twin Sample: The Brainscale Study. *Cereb Cortex.* 2018.
19. Gielen M, van Beijsterveldt CE, Derom C, Vlietinck R, Nijhuis JG, Zeegers MP, Boomsma DI. Secular trends in gestational age and birthweight in twins. *Hum Reprod.* 2010;25(9):2346-2353.

20. Scheet P, Ehli EA, Xiao X, van Beijsterveldt CE, Abdellaoui A, Althoff RR, Hottenga JJ, Willemsen G, Nelson KA, Huizenga PE, Hu Y, Amos CI, Bartels M, Groen-Blokhuis MM, de Geus EJ, Hudziak JJ, Davies GE, Boomsma DI. Twins, tissue, and time: an assessment of SNPs and CNVs. *Twin Res Hum Genet.* 2012;15(6):737-745.
21. Homer N, Kothiya S, Rutter A, Walker BR, Andrew R. Gas chromatography tandem mass spectrometry offers advantages for urinary steroids analysis. *Anal Biochem.* 2017;538:34-37.
22. Odintsova VV, Willemsen G, Dolan CV, Hottenga JJ, Martin NG, Slagboom PE, Ordonana JR, Boomsma DI. Establishing a Twin Register: An Invaluable Resource for (Behavior) Genetic, Epidemiological, Biomarker, and 'Omics' Studies. *Twin Res Hum Genet.* 2018;21(3):239-252.
23. Lindstrom ML, Bates DM. Nonlinear mixed effects models for repeated measures data. *Biometrics.* 1990;46(3):673-687.
24. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics.* 1982;38(4):963-974.
25. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nat Rev Genet.* 2002;3(11):872-882.
26. Boker S, Neale M, Maes H, Wilde M, Spiegel M, Brick T, Spies J, Estabrook R, Kenny S, Bates T, Mehta P, Fox J. OpenMx: An Open Source Extended Structural Equation Modeling Framework. *Psychometrika.* 2011;76(2):306-317.
27. Finken MJ, van der Voorn B, Heijboer AC, de Waard M, van Goudoever JB, Rotteveel J. Glucocorticoid Programming in Very Preterm Birth. *Horm Res Paediat.* 2016;85(4):221-231.
28. TNO. Vijfde Landelijke Groeistudie. 2010.
29. Neumann A, Direk N, Crawford AA, Mirza S, Adams H, Bolton J, Hayward C, Strachan DP, Payne EK, Smith JA, Milanesechi Y, Penninx B, Hottenga JJ, de Geus E, Oldehinkel AJ, van der Most PJ, de Rijke Y, Walker BR, Tiemeier H. The low single nucleotide polymorphism heritability of plasma and saliva cortisol levels. *Psychoneuroendocrinology.* 2017;85:88-95.
30. Ross KM, Murphy ML, Adam EK, Chen E, Miller GE. How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. *Psychoneuroendocrinology.* 2014;39:184-193.
31. van Bodegom M, Homberg JR, Henckens M. Modulation of the Hypothalamic-Pituitary-Adrenal Axis by Early Life Stress Exposure. *Front Cell Neurosci.* 2017;11:87.
32. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry.* 2001;49(12):1023-1039.
33. Francis DD, Champagne FA, Liu D, Meaney MJ. Maternal care, gene expression, and the development of individual differences in stress reactivity. *Ann N Y Acad Sci.* 1999;896:66-84.
34. de Kloet ER, Sibug RM, Helmerhorst FM, Schmidt MV. Stress, genes and the mechanism of programming the brain for later life. *Neurosci Biobehav Rev.* 2005;29(2):271-281.
35. McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci.* 2009;12(3):342-348.
36. Lester BM, Conratt E, LaGasse LL, Tronick EZ, Padbury JF, Marsit CJ. Epigenetic Programming by Maternal Behavior in the Human Infant. *Pediatrics.* 2018;142(4).
37. Roth TL, Zoladz PR, Sweatt JD, Diamond DM. Epigenetic modification of hippocampal Bdnf DNA in adult rats in an animal model of post-traumatic stress disorder. *J Psychiatr Res.* 2011;45(7):919-926.
38. Dirven BCJ, Homberg JR, Kozicz T, Henckens M. Epigenetic programming of the neuroendocrine stress response by adult life stress. *J Mol Endocrinol.* 2017;59(1):R11-R31.
39. Sipahi L, Wildman DE, Aiello AE, Koenen KC, Galea S, Abbas A, Uddin M. Longitudinal epigenetic variation of DNA methyltransferase genes is associated with vulnerability to post-traumatic stress disorder. *Psychol Med.* 2014;44(15):3165-3179.
40. Franz CE, York TP, Eaves LJ, Mendoza SP, Hauger RL, Hellhammer DH, Jacobson KC, Levine S, Lupien SJ, Lyons MJ, Prom-Wormley E, Xian H, Kremen WS. Genetic and environmental



- influences on cortisol regulation across days and contexts in middle-aged men. *Behav Genet.* 2010;40(4):467-479.
41. Lucassen PJ, Naninck EFG, van Goudoever JB, Fitzsimons C, Joels M, Korosi A. Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics. *Trends Neurosci.* 2013;36(11):621-631.
  42. Hng TM, Cheung NW, McLean M. Growth hormone and cortisol dynamic function in relation to birth weight: a study in adult twins. *J Clin Endocrinol Metab.* 2005;90(5):2781-2786.
  43. Finken M. Glucocorticoid-Programming in Very Preterm Birth. *Ann Nutr Metab.* 2016;69(2):103-103.
  44. Marsman R, Nederhof E, Rosmalen JG, Oldehinkel AJ, Ormel J, Buitelaar JK. Family environment is associated with HPA-axis activity in adolescents. The TRAILS study. *Biol Psychol.* 2012;89(2):460-466.
  45. Silventoinen K, Haukka J, Dunkel L, Tynelius P, Rasmussen F. Genetics of pubertal timing and its associations with relative weight in childhood and adult height: the Swedish Young Male Twins Study. *Pediatrics.* 2008;121(4):e885-891.
  46. Cheung C, Yu AM, Chen CS, Krausz KW, Byrd LG, Feigenbaum L, Edwards RJ, Waxman DJ, Gonzalez FJ. Growth hormone determines sexual dimorphism of hepatic cytochrome P450 3A4 expression in transgenic mice. *J Pharmacol Exp Ther.* 2006;316(3):1328-1334.
  47. Low SC, Chapman KE, Edwards CR, Wells T, Robinson IC, Seckl JR. Sexual dimorphism of hepatic 11 beta-hydroxysteroid dehydrogenase in the rat: the role of growth hormone patterns. *J Endocrinol.* 1994;143(3):541-548.
  48. Federenko IS, Nagamine M, Hellhammer DH, Wadhwa PD, Wust S. The heritability of hypothalamus pituitary adrenal axis responses to psychosocial stress is context dependent. *J Clin Endocrinol Metab.* 2004;89(12):6244-6250.
  49. Schreiber JE, Shirtcliff E, Van Hulle C, Lemery-Chalfant K, Klein MH, Kalin NH, Essex MJ, Goldsmith HH. Environmental influences on family similarity in afternoon cortisol levels: twin and parent-offspring designs. *Psychoneuroendocrinology.* 2006;31(9):1131-1137.
  50. Ouellet-Morin I, Boivin M, Dionne G, Lupien SJ, Arseneault L, Barr RG, Perusse D, Tremblay RE. Variations in heritability of cortisol reactivity to stress as a function of early familial adversity among 19-month-old twins. *Arch Gen Psychiatry.* 2008;65(2):211-218.
  51. Steptoe A, van Jaarsveld CH, Semmler C, Plomin R, Wardle J. Heritability of daytime cortisol levels and cortisol reactivity in children. *Psychoneuroendocrinology.* 2009;34(2):273-280.
  52. Riese H, Rijdsdijk FV, Rosmalen JG, Snieder H, Ormel J. Neuroticism and morning cortisol secretion: both heritable, but no shared genetic influences. *J Pers.* 2009;77(5):1561-1575.
  53. Van Hulle CA, Shirtcliff EA, Lemery-Chalfant K, Goldsmith HH. Genetic and environmental influences on individual differences in cortisol level and circadian rhythm in middle childhood. *Horm Behav.* 2012;62(1):36-42.
  54. Rietschel L, Streit F, Zhu G, McAloney K, Kirschbaum C, Frank J, Hansell NK, Wright MJ, McGrath JJ, Witt SH, Rietschel M, Martin NG. Hair Cortisol and Its Association With Psychological Risk Factors for Psychiatric Disorders: A Pilot Study in Adolescent Twins. *Twin Res Hum Genet.* 2016;19(5):438-446.
  55. Tucker-Drob EM, Grotzinger AD, Briley DA, Engelhardt LE, Mann FD, Patterson M, Kirschbaum C, Adam EK, Church JA, Tackett JL, Harden KP. Genetic influences on hormonal markers of chronic hypothalamic-pituitary-adrenal function in human hair. *Psychol Med.* 2017:1-13.
  56. Rietschel L, Streit F, Zhu G, McAloney K, Frank J, Couvy-Duchesne B, Witt SH, Binz TM, Consortium CO, Major Depressive Disorder Working Group of the Psychiatric Genomics C, McGrath J, Hickie IB, Hansell NK, Wright MJ, Gillespie NA, Forstner AJ, Schulze TG, Wust S, Nothen MM, Baumgartner MR, Walker BR, Crawford AA, Colodro-Conde L, Medland SE, Martin NG, Rietschel M. Hair Cortisol in Twins: Heritability and Genetic Overlap with Psychological Variables and Stress-System Genes. *Sci Rep.* 2017;7(1):15351.

**Figure 1** This flowchart presents the enrollment of participants for this study

**Figure 2** Path diagram representing the ACE (top) and ADE (bottom) model.

Accepted Manuscript

**Table 1** Summary table on previous cortisol heritability twin studies (published after the meta-

First author	Publication	Medium	Type	Sample size (n twins)	Sex	Age	Heritability (%)
Bartels*(7)	2003	Saliva & plasma	Sampling across the day	798 (418 MZ, 380 DZ)	Males & Females	Children & adults	62
Bartels (10)	2003	Saliva	Awakening, 30-60 min post awakening, 12.30h, 20.30h	360	Males & Females	Children (12y)	22-24, 56-59, 30-21, 0
Federenko (48)	2004	Saliva & plasma	After stress exposure (3 times, salivary and total cortisol)	116 (66 MZ, 50 DZ)	Males	Children & adults (16-24y)	8, 56, 100, 32, 36, 98
Kupper (9)	2005	Saliva	Awakening, 30 min post awakening and evening	471 (199 MZ, 272 DZ)	Males & Females	Adults	34, 32, 0
Schreiber (49)	2006	Saliva	Prior to dinner	412 (176 MZ, 236 DZ)	Males & Females	Children(8y)	0
Ouellet-Morin(50)	2008	Saliva	Cortisol reactivity	346 (130 MZ, 216 DZ)	Males & Females	Children (19mo)	51
Steptoe (51)	2009	Saliva	Cortisol reactivity	150 (80 MZ, 70 DZ)	Males & Females	Children (11y)	58, 60, 56, 44
Riese (52)	2009	Saliva	Awakening, 30 45 and 60 min post awakening	250 (184 MZ, 102 DZ)	Females	Adults	46, 69, 69, 52
Franz (40)	2010	Saliva	Awakening, 30 min post awakening, 10.00h, mean output across the day, mean CAR	401 (196 MZ, 205 DZ)	Males	Adults	56, 48, 42, 43, 64
Gustafsson (8)	2011	Saliva	Awakening, 30 min post awakening, bedtime	302 (154 MZ, 148 DZ)	Males & Females	Children (9-16y)	28, 60, 8
Van Hulle (53)	2012	Saliva	Awakening, slope, evening level	904 (314 MZ, 578 DZ)	Males & Females	Children (7-8y)	31, 32, 0
Ouellet-Morin (11)	2016	Saliva	Awakening, CAR, diurnal change	592 (280 MZ, 312 DZ)	Males & Females	Children (14y)	28, 50, 31

analyses in 2003 of Bartels et al)

Rietschel (54)	2016	Hair cortisol	3 cm hair	58 (16 MZ, 42 DZ)	Males & Females	Children & young adults (15y)	0
Tucker-Drob (55)	2017	Hair cortisol	3 cm hair	1214 (376 MZ, 838 DZ)	Males & Females	Children (8-20yjr)	65
Rietschel (56)	2017	Hair cortisol	3 cm hair	671 (232 MZ, 374 DZ, trizygotic twins)	Males & Females	Children (15y)	72

\* This study included an analysis of five original, comparable twin studies focusing specifically on basal cortisol levels (Wust et al. 2000, Froehlich et al. 2000, Inglis et al. 1999, Linkowski et al.1993, and Meikle et al., 1988)

The numbers presented in this table were extracted from (7-11,40,48-56)

Accepted Manuscript

**Table 2** Ratios indicating enzyme activity

Urine metabolites	Index	9yr median [IQR]	12 yr median [IQR]	17 yr median [IQR]
(THF + allo-THF + THE + $\alpha$ -cortol + $\beta$ -cortol + $\alpha$ -cortolone + $\beta$ -cortolone)/creatinine	Sum of cortisol metabolites (cortisol production rate)	0.5666 [0.29]	0.5374 [0.28]	0.3777 [0.30]
allo-THF/cortisol	5 $\alpha$ -reductase activity	8.2273 [7.55]	6.9109 [6.19]	5.0453 [6.85]
THF/cortisol	5 $\beta$ -reductase activity (a)	8.0941 [7.43]	9.6624 [7.23]	7.8718 [31.42]
THE/cortisone	5 $\beta$ -reductase activity (b)	23.8426 [13.94]	25.1619 [16.93]	24.5388 [17.03]
cortisol/cortisone	Renal 11 $\beta$ -HSD type 2 activity	0.7290 [0.62]	0.6677 [0.41]	1.1074 [0.66]
(THF + allo-THF)/THE	Global 11 $\beta$ -HSD activities	0.6079 [0.43]	0.5109 [0.36]	0.6176 [0.40]
6-OH cortisol/cortisol	Cytochrome P450 3A4 activity	1.5363 [1.21]	1.5531 [1.44]	1.6866 [1.02]

Abbreviations: IQR, interquartile range; THF, tetrahydrocortisol; THE, tetrahydrocortisone, HSD, hydroxysteroid dehydrogenase

**Table 3** Intraclass correlations of batch corrected data with fixed covariates sex and gestational age (\* p<0.05, \*\* p<0.01).

Phenotype	Age (y)	Number (n)	MZ		DZ		Model	b0	bsex	bGA
			ICC	Variance	ICC	Variance				
Cortisol production rate	9	167	0.294	0.031	0.037	0.054	ADE	-0.357	-0.014	0.016
	12	147	0.358	0.059	0.021	0.039	ADE	0.142	-0.017	-0.004
	17	148	0.055	0.056	0.307	0.055	ACE	0.523	0.047	-0.015
5 $\alpha$ -reductase activity	9	188	0.547	30.448	0.467	25.942	ACE	-24.209	0.075	0.656
	12	141	0.749	18.898	0.522	18.869	ACE	-2.467	-1.927*	0.094
	17	151	0.489	12.039	0.400	19.728	ACE	-0.180	3.179**	-0.036
5 $\beta$ -reductase activity (a)	9	190	0.466	21.215	0.459	21.320	ACE	-7.148	0.421	0.189
	12	156	0.000	23.388	0.245	35.027	ACE	3.263	0.238	-0.094
	17	153	0.645	13.693	0.062	13.402	ADE	-1.795	1.178	-0.036
5 $\beta$ -reductase activity (b)	9	204	0.691	94.811	0.452	62.492	ACE	11.922	-0.453	-0.314
	12	165	0.548	128.790	0.120	102.764	ADE	2.128	-2.269	-0.028
	17	156	0.565	91.055	0.400	201.990	ACE	-18.345	6.839**	0.444
Renal 11 $\beta$ -HSD type 2 activity	9	195	0.206	0.120	0.000	0.119	ADE	-0.526	0.005	0.014
	12	159	0.640	0.056	0.228	0.067	ADE	-1.344	0.064	0.036*
	17	155	0.220	0.154	0.305	0.186	ACE	-1.195	-0.102	0.036
Global 11 $\beta$ -HSD activities	9	197	0.548	0.048	0.383	0.029	ACE	-1.214	0.038	0.032*
	12	162	0.246	0.076	0.043	0.030	ADE	-0.530	0.047	0.014
	17	153	0.123	0.048	0.166	0.056	ACE	-0.514	0.068	0.014
Cytochrome P450 3A4 activity	9	185	0.275	0.686	0.215	0.473	ACE	-1.574	0.066	0.044
	12	144	0.626	1.486	0.233	0.628	ADE	1.004	-0.080	-0.028
	17	148	0.427	0.318	0.343	0.670	ACE	1.286	0.078	-0.036

Abbreviations: MZ, monozygotic; DZ, dizygotic; ICC, intraclass correlation coefficient; HSD, hydroxysteroid dehydrogenase; b0, intercept; bsex, regression coefficient sex; bGA, regression coefficient gestational age

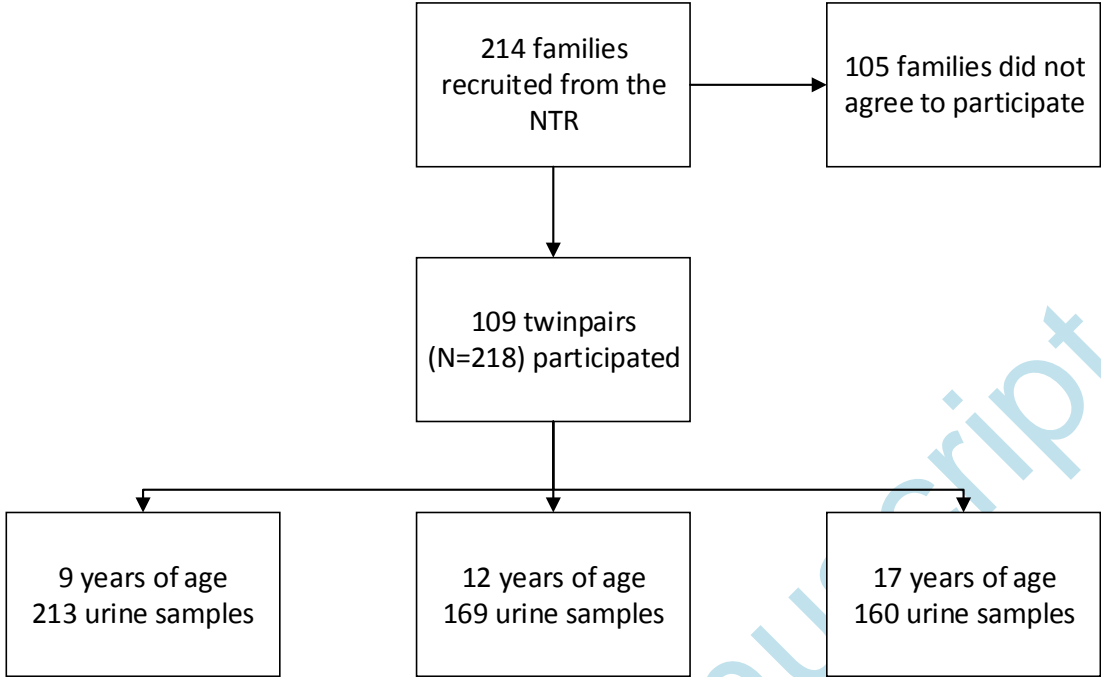
Accepted Article

**Table 4** Estimates of genetic (A, D) and environmental (C, E), raw and standardized, 95% Confidence intervals in parentheses.

Index		Age (yr)	A (additive genetic)	C (shared environment)	D (dominance)	E (unshared environment)	Broad-sense heritability (A+D)
Cortisol production rate	Raw	9	0.000	-	0.019	0.027	-
		12	0.000	-	0.014	0.033	-
		17	0.000	0.012	-	-	0.044
	Standardized	9	0.000 (0.00-0.56)	-	0.415 (0.00-0.69)	0.585 (0.31-1.00)	42%
		12	0.000 (0.00-0.51)	-	0.301 (0.00-0.54)	0.699 (0.46-1.00)	30%
		17	0.000 (0.00-0.41)	0.210 (0.00-0.42)	-	0.790 (0.57-1.00)	0%
5 $\alpha$ -reductase activity	Raw	9	4.163	10.568	-	12.827	-
		12	8.562	5.579	-	4.739	-
		17	8.574	1.650	-	6.571	-
	Standardized	9	0.151 (0.00-0.68)	0.384 (0.00-0.63)	-	0.466 (0.29-0.69)	15%
		12	0.454 (0.00-0.84)	0.296 (0.00-0.67)	-	0.251 (0.14-0.46)	45%
		17	0.511 (0.00-0.78)	0.098 (0.00-0.57)	-	0.391 (0.22-0.72)	51%
5 $\beta$ -reductase activity (a)	Raw	9	0.000	9.646	-	11.176	-
		12	0.000	3.236	-	26.233	-
		17	0.000	-	8.612	4.963	-
	Standardized	9	0.000 (0.00-0.50)	0.463 (0.00-0.61)	-	0.537 (0.37-0.72)	0%
		12	0.000 (0.00-0.00)	0.110 (0.00-0.32)	-	0.890 (0.67-1.00)	0%
		17	0.000 (0.00-0.71)	-	0.634 (0.00-0.78)	0.366 (0.22-0.62)	63%
5 $\beta$ -reductase activity (b)	Raw	9	17.713	30.521	-	27.502	-
		12	3.856	-	53.255	55.417	-
		17	90.367	0.000	-	46.988	-
	Standardized	9	0.234 (0.00-0.72)	0.403 (0.00-0.68)	-	0.363 (0.24-0.54)	23%
		12	0.034 (0.00-0.66)	-	0.473 (0.00-0.69)	0.493 (0.31-0.76)	51%
		17	0.658 (0.14-0.81)	0.000 (0.00-0.31)	-	0.342 (0.19-0.64)	66%
Renal 11 $\beta$ -hydroxysteroid dehydrogenase type 2 activity	Raw	9	0.000	-	0.014	0.106	-
		12	0.012	-	0.032	0.021	-
		17	0.000	0.047	-	0.125	-
	Standardized	9	0.000 (0.00-0.27)	-	0.113 (0.00-0.39)	0.887 (0.61-1.00)	11%
		12	0.185 (0.00-0.79)	-	0.494 (0.00-0.81)	0.321 (0.19-0.58)	68%
		17	0.000 (0.00-0.54)	0.274 (0.00-0.47)	-	0.726 (0.46-0.95)	0%
Global 11 $\beta$ -hydroxysteroid dehydrogenase activities	Raw	9	0.000	0.018	-	0.020	-
		12	0.009	-	0.000	0.040	-
		17	0.000	0.008	-	0.044	-
	Standardized	9	0.000 (0.00-0.59)	0.476 (0.00-0.62)	-	0.524 (0.36-0.70)	0%
		12	0.183 (0.00-0.39)	-	0.000 (0.00-0.39)	0.817 (0.61-1.00)	18%
		17	0.000 (0.00-0.47)	0.151 (0.00-0.36)	-	0.849 (0.53-1.00)	0%
Cytochrome P450 3A4 activity	Raw	9	0.233	0.000	-	0.373	-
		12	0.206	-	0.527	0.360	-
		17	0.327	0.000	-	0.211	-
	Standardized	9	0.385 (0.00-0.62)	0.000 (0.00-0.40)	-	0.616 (0.38-0.96)	39%
		12	0.188 (0.00-0.81)	-	0.482 (0.00-0.82)	0.330 (0.18-0.66)	67%
		17	0.608 (0.00-0.78)	0.000 (0.00-0.48)	-	0.393 (0.22-0.75)	61%

\* Broad-sense heritability is the degree to which a trait is genetically determined (additive + non-additive genetic effects)

**Figure 1** This flowchart presents the enrollment of participants for this study



Accepted Manuscript



**Figure 2** Path diagram representing the ACE (top) and ADE (bottom) model.

