Journal of Psychiatric Research 176 (2024) 68–76

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

The relationship between hair cortisol concentration and autism diagnosis

Ping-I Lin [a,b], James Rufus John [a,c], Anne Masi [a], Lin Kooi Ong [d,e,f,g], Nisha E. Mathew [h], Mohammed Ali Moni [i], Valsamma Eapen [a,c,j], Adam K. Walker [a,h,k,*]

[a] Discipline of Psychiatry and Mental Health, School of Clinical Medicine, University of New South Wales, Sydney, NSW, Australia
[b] Department of Psychiatry and Behavioral Neuroscience, Saint Louis University School of Medicine, MO, USA
[c] Ingham Institute of Applied Medical Research, Liverpool, NSW, Australia
[d] School of Health and Medical Sciences & Centre for Health Research, University of Southern Queensland, Toowoomba, QLD, 4350, Australia
[e] School of Pharmacy, Monash University Malaysia, Selangor, Malaysia
[f] School of Biomedical Sciences and Pharmacy, The University of Newcastle, Callaghan, NSW, Australia
[g] Heart and Stroke Research Program, Hunter Medical Research Institute, New Lambton Heights, NSW, Australia
[h] Laboratory of ImmunoPsychiatry, Neuroscience Research Australia, Randwick, NSW, Australia
[i] School of Health and Rehabilitation Sciences, The University of Queensland, QLD, Australia
[j] Academic Unit of Child and Adolescent Psychiatry, South Western Sydney Local Health District (AUCS), Liverpool, NSW, Australia
[k] Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Parkville, VIC, Australia

ARTICLE INFO

Keywords:
Autism
Hair cortisol
Chronic stress
Family income
Age
Residence

ABSTRACT

Background: Autistic children are prone to experience heightened levels of distress and physiological reactivity to a range of sensory, social, and emotional stimuli. In line with this, multiple studies have demonstrated that autistic children have higher acute cortisol stress responses to adverse or threatening stimuli and altered cortisol awakening responses. However, few studies have examined whether this sensitivity may relate to heightened levels of chronic stress and persistently elevated hypothalamic-pituitary-adrenal (HPA) axis activity. The measurement of cortisol accumulation in hair is considered a non-invasive biomarker of chronic stress and has been associated with several childhood diseases. Here, we investigated whether hair cortisol concentration in a large sample of autistic children differed from non-autistic children, and after accounting for a range of child, parental and family-level characteristics.

Methods: Hair cortisol concentration was measured in 307 autistic children and 282 non-autistic controls aged between 2 and 17 years recruited from four Australian states who participated in providing hair samples and demographic data to the Australian Autism Biobank. Independent samples *t*-test or one-way analysis of variance (ANOVA) were conducted to determine significant differences in the mean hair cortisol concentration (pg/mg) between potential covariates. Primary analysis included multivariable regression modelling of the collapsed sample to identify variables that were significantly associated with hair cortisol concentration after controlling for covariates. We also accounted for the potential interaction of multiple biological (e.g., age, sex, BMI) and psychosocial characteristics at the level of the child, the mother and the father, and the family unit.

Results: Our findings suggest that the diagnosis of autism was not a significant predictor of chronic stress, as measured by hair cortisol concentration. However, findings of the multivariable regression analysis showed that key factors such as area of residence (Queensland vs Victorian state of residence) and decrease in child's age were significantly associated with higher hair cortisol concentration whereas lower family income was significantly associated with higher hair cortisol concentration.

Conclusion: To our knowledge, this is the first study to show that socioeconomic factors such as family annual income affect hair cortisol status in autistic children, indicating that the psychosocial environment may be a potential mediator for chronic stress in autistic children just as it has been demonstrated in non-autistic children.

* Corresponding author. Laboratory of ImmunoPsychiatry, Neuroscience Research Australia, 139 Barker Street, Randwick, NSW, 2031, Australia.
E-mail address: a.walker@neura.edu.au (A.K. Walker).

https://doi.org/10.1016/j.jpsychires.2024.05.052
Received 3 May 2023; Received in revised form 18 April 2024; Accepted 29 May 2024
Available online 30 May 2024

1. Introduction

Autism Spectrum Disorder (ASD) is a highly heterogeneous neurodevelopmental disorder characterised by impairments in social communication and the presence of restricted, repetitive behaviours and interests (American Psychiatric Association, 2013). Difficulties in social communication and interactions, such as understanding nonverbal cues, initiating or maintaining conversations, or interpreting social situations, can lead to misunderstandings, social isolation, and difficulty forming relationships, which can be stressful. Autistic children are prone to experience heightened levels of distress and physiological reactivity to a range of sensory, social, and emotional stimuli (Lydon et al., 2016). These emotional and physiological responses may be attributed to underlying sensitivity to sensory stimulation or psychosocial events. Co-occurring physical health and mental health issues may also contribute to increased stress levels in autistic individuals and exacerbate the severity of autism traits (Lai et al., 2019; Tye et al., 2019). The hypothalamic-pituitary-adrenal (HPA) axis is a well-known biological stress pathway that can impact various aspects of health, including behavior, gut function, and sleep (e.g. Kennedy et al., 2014). The dysregulation of this pathway suggests that stress may be a critical factor in regulating co-occurring conditions that contribute to behavioral disturbances in autistic children. Unfortunately, a substantial proportion of autistic children are unable to verbalise their distress due to limitations in communicative skills and cognitive capacity (Tager-Flusberg and Kasari, 2013; Maenner et al., 2023). Parental reporting of stress and parental perceptions of stress and quality of life may be impacted by a wide range of factors (Eapen et al., 2022) independent of the child's stress levels and hence may have lesser utility. Further, subjectively reported experiences may not always align with the biological parameters of stress (Faurholt-Jepsen et al., 2021) and should not be considered a substitute for measuring cortisol or other stress hormones to understand HPA axis (dys)regulation. Hence, there is a critical need to identify appropriate biological markers of stress.

The most common biological stress assessment involves measuring cortisol levels. Accumulating evidence indicates that measurement of cortisol in the blood, urine, and saliva of autistic children can provide a quantifiable, objective measure of distress. However, studies on this topic have produced inconsistent results, possibly due to small sample sizes, participant avoidance of needle sticks or mouth swabs, and the limited analytic power resulting from the unbalanced male to female ratio of approximately 1:3 in autism (Ratto et al., 2018; Saxbe et al., 2017; Sharpley et al., 2016; Spratt et al., 2012). Further, two systematic reviews examining HPA axis function in autism and Fragile X syndrome, a common genetic cause of autism, have revealed significant heterogeneity in the assessment of HPA axis activation in biological fluids among autistic children (Hadwin et al., 2019; Hardiman and Bratt, 2016). Such heterogeneity is further compounded by the fact that the cortisol levels in biological fluids are vulnerable to changes in relation to acute events of distress and affected by the time of the day of sample collection due to the diurnal variation (Chung et al., 2011). Together, these issues mean that single time point analyses are relatively unreliable to indicate chronic stress. While it is possible to collect multiple samples across time, the practical considerations and participant burden mean this is typically not feasible. An ideal approach for assessing stress levels would be a measure that is stable over an extended time frame (weeks to months), does not involve self-recall, and is not biased by inherent biological pulsatility.

Emerging evidence suggests that analyzing cortisol levels in hair can offer a reliable indication of chronic cortisol levels of up to 6 months (Stalder and Kirschbaum, 2012; Vives et al., 2015). Considering this, we conducted an exploratory study comparing hair cortisol concentrations between the largest group of autistic children, non-autistic siblings and unrelated non-autistic children to date. Additionally, we explored the potential use of hair cortisol in non-autistic siblings as a biomarker for chronic familial stress in households with one or more autistic children; the assumption being that the presence of an autistic child in the family would influence hair cortisol concentration relationships with predictor variables in non-autistic siblings differently to unrelated non-autistic children.

2. Methods

2.1. Participants

Participants in this study came from the Australian Autism Biobank comprising autistic children and adolescents, their non-autistic siblings and unrelated non-autistic controls without a diagnosis of ASD and no first-degree autistic relatives (Alvares et al., 2018). Details of consent and recruitment procedures are available in the original reports (Alvares et al., 2018). All participants who provided a useable hair sample during recruitment were included in this study (N = 589). All experimental procedures and analyses in this paper were approved by the University of New South Wales Human Research Ethics Committee (HC200078) and permission to use the sample was obtained from Autism CRC (2020017_CSC).

2.2. Clinical and behavioural measures

Autistic traits were assessed using the Autism Diagnostic Observation Schedule (ADOS) (2nd edition (Hus and Lord, 2014). The ADOS is a semi-structured, standardized assessment of autism-linked differences in communication, social interaction, and restricted and repetitive behaviours. Higher calibrated severity scores reflect greater levels of autism-associated traits after accounting for age and language ability. For analysis, we dichotomised the sample as autistic participants and those without a diagnosis (siblings and controls).

2.3. Hair sample analysis

Hair processing and cortisol measurement was conducted by Stratech Scientific (Mona Vale, Australia) according to validated protocols (e.g. Bryson et al., 2019). Briefly, hair samples were assessed and cut into 4 cm lengths using the end of the hair closest to the scalp where possible. Hair samples were then treated with cleaning solvent and allowed to dry for 5 days. After 5 days, the hair was weighed for extraction and mechanically reduced. Extraction solvent was used for 24 h with sonication and tubes were dried to remove all solvent. Samples were then reconstituted in PBS for analysis. Cortisol concentrations were analysed in duplicate using a commercially available ELISA assay (Salimetrics, USA) according to the manufacturer's instructions. The assay technician was blind to all details of participant characteristics when assaying samples including the diagnostic status of the sample. The mean coefficient of variation between duplicates was <10.1 % and the mean intra-assay coefficient of variance was <11 %.

2.4. Statistical analysis

All statistical analyses were conducted using R Studio (Version 1.4.1564 on MacOS 10.15.7). Descriptive statistics were conducted to report on baseline characteristics of participants using mean and standard deviation (SD) and percentages for categorical measures. Independent samples *t*-test or one-way analysis of variance (ANOVA) were conducted to determine significant differences in the mean hair cortisol concentration (pg/mg) between potential covariates. Since the distribution of the outcome variable (hair cortisol concentration) was highly skewed, we used rank-based inverse normal transformation to normalise the variable to alleviate inflated type-I error (McCaw et al., 2020). Primary analysis included univariable and multivariable linear regression modelling to identify variables that were significantly associated with hair cortisol concentration after controlling for covariates. All variables with p-value <0.25 were included in the multivariable model. The full

model was reduced using backward stepwise regression to remove non-significant variables and only the variables that were statistically associated ($p < 0.05$) with the outcome variable remained in the final model.

3. Results

3.1. Characteristics of participants by diagnosis of Autism Spectrum Disorder

The study population comprised hair cortisol data from 307 autistic participants and 282 non-autistic participants. Comparison of demographic characteristics between autistic participants and non-autistic participants revealed several expected and unexpected differences between these groups (Table 1). The age distribution of autistic participants significantly differed compared to the non-autistic control group ($p = 0.01$). Reflective of 5 years being the mean age of autism diagnosis (van't Hof et al., 2021), the autistic group had a smaller proportion of participants under 5 years old and a higher proportion between 6 and 12 years than the non-autistic control group. The proportion of participants between 13 and 17 years were similar between both groups. The gender distribution between the groups also significantly differed ($p < 0.001$) with 3.32 fold more males in the autism group which is consistent with the 1:3 male to female ratio in autism while the sex distribution in the non-autism group was almost evenly split. While English was spoken at home in most households, the autism group had a significantly higher number of children whose household language was not English compared to the non-autism group ($p = 0.011$). There was a significantly higher proportion of children with other comorbid conditions in the autistic group than the non-autistic group ($p < 0.001$).

Maternal and paternal characteristics differed between the autism and non-autism groups (Table 1). The mean age values of both parents were significantly higher for the autism group than the non-autism group ($p < 0.001$ for both). There was a significantly higher proportion of both mothers ($p = 0.002$) and fathers ($p = 0.009$) born outside of Australia in the non-autism group compared to the autism group. There was a higher proportion of non-Caucasian mothers in the autism group ($p = 0.01$) but there was no proportional difference for fathers. The highest level of education was significantly higher for mothers and fathers of children in the autism group ($p \leq 0.01$ for both). The employment status of fathers but not mothers significantly differed between groups with more fathers in the autism group being professionals ($p < 0.01$). Household income did not differ between the groups ($p = 0.66$).

3.2. Hair cortisol concentrations by child and parent-level characteristics

The mean hair cortisol concentration did not differ between autistic children and non-autistic children without adjusting for other factors (Table 1). To explore whether the child's or parents' characteristics may moderate the relationship between hair cortisol concentrations and the diagnosis of autism, we compared hair cortisol concentrations collapsed by diagnostic group among key characteristics of children and parents (Table 2). When data were collapsed across diagnostic groups, a number of child or parent level characteristics significantly affected hair cortisol concentrations. Children aged under 5 years had significantly higher hair cortisol than children 5 years and older ($p < 0.001$). Notably, there was an age-dependent change in hair cortisol concentration, such that, hair cortisol successively reduced as children got older across the three age categories. Children of mothers born outside of Australia had a significantly higher hair cortisol level ($p = 0.045$). Child's hair cortisol concentration significantly increased as family annual income got lower with children of families earning less than $70,000 having the highest hair cortisol concentration ($p = 0.005$). The mean hair cortisol concentration significantly differed based on the location of recruitment ($p = 0.05$), such that the mean cortisol concentration of participants in Western Australia was higher than the other recruitment sites.

Table 1
Characteristics of participants by diagnosis of Autism Spectrum Disorder.

Characteristics	Autistic Participants (Proband) (N = 307)	Non-autistic Participants (Controls and siblings) (N = 282)	p-value
Child-level characteristics			
Hair cortisol, mean pg/mg (SD)	9.66 (18.51)	8.50 (13.07)	0.377
Age in years			
Up to 5 years	95 (30.9)	121 (42.9)	**0.010**
6-12 years	175 (57.0)	131 (46.5)	
13-17 years	37 (12.1)	30 (10.6)	
Gender			
Male	236 (76.9)	136 (48.2)	**<0.001**
Female	71 (23.1)	146 (51.8)	
Do they have a twin?			
No	285 (93.4)	196 (93.8)	0.878
Yes	20 (6.6)	13 (6.2)	
More than one autistic child in immediate family			
No	175 (61.8)	3 (100.0)	0.175
Yes	108 (38.2)	0 (0.0)	
Child's BMI, mean (SD)	17.9 (4.1)	17.5 (3.3)	0.193
Tanner stage			
Before puberty	80 (71.4)	49 (67.1)	0.533
During puberty	32 (28.6)	24 (32.9)	
Type of usual residence			
Parental home	280 (98.9)	225 (98.7)	0.790
Other residence	3 (1.1)	3 (1.3)	
Language spoken at home			
English	263 (93.6)	223 (98.2)	**0.011**
Other	18 (6.4)	4 (1.8)	
Other conditions			
No	173 (56.4)	235 (83.3)	**<0.001**
Yes	134 (43.6)	47 (16.7)	
Total number of medications, mean (SD)	1.1 (0.9)	1.0 (0.8)	0.460
ADOS-2 score	6.8 (1.9)	–	–
Maternal characteristics			
Mother's age, mean (SD)	39.8 (6.3)	34.2 (8.2)	**<0.001**
Mother's country of birth			
Australia	171 (55.7)	121 (42.9)	**0.002**
Other	136 (44.3)	161 (57.1)	
Mother's ethnicity			
Caucasian	187 (76.3)	124 (86.7)	**0.013**
Other	58 (23.7)	19 (13.3)	
Mother's education level			
High school level	52 (21.1)	52 (36.1)	**0.002**
Trade, certificate, other levels	75 (30.4)	43 (29.9)	
University degree	120 (48.6)	49 (34.0)	
Mother's employment status			
Home duties	50 (20.6)	28 (20.0)	0.893
Professional or skilled labour	193 (79.4)	112 (80.0)	
Mother's BMI, mean (SD)	27.8 (6.5)	28.1 (7.1)	0.615
Mother's smoking status during pregnancy			
No	227 (81.4)	–	–
Yes	52 (18.6)	–	
Mother's drinking status during pregnancy			
No	81 (28.9)	–	–
Yes	199 (71.1)	–	
Paternal characteristics			
Father's age, mean (SD)	42.9 (7.2)	36.2 (8.6)	**<0.001**
Father's country of birth			
Australia	158 (51.5)	114 (40.7)	**0.009**
Other	149 (48.5)	166 (59.3)	
Father's ethnicity			
Caucasian	199 (81.6)	118 (83.7)	0.597
Other	45 (18.4)	23 (16.3)	
Father's education level			
High school level	65 (27.2)	45 (32.1)	**0.005**

(*continued on next page*)

Table 1 (*continued*)

Characteristics	Autistic Participants (Proband) (N = 307)	Non-autistic Participants (Controls and siblings) (N = 282)	p-value
Trade, certificate, other levels	79 (33.1)	62 (44.3)	
University degree	95 (39.7)	33 (23.6)	
Father's employment status			
Professional	118 (52.4)	39 (30.0)	**<0.001**
Skilled labour	107 (47.6)	91 (70.0)	
Father's BMI, mean (SD)	28.0 (4.8)	28.2 (4.7)	0.789
Father's smoking status during pregnancy			
No	195 (75.3)	–	-
Yes	64 (24.7)	–	
Father's drinking status during pregnancy			
No	46 (17.6)	–	–
Yes	215 (82.4)	–	
Family annual income			
Up to AUD 70,000 per year	62 (28.3)	45 (32.6)	0.656
AUD 70,001 – 104,000 per year	66 (30.1)	41 (29.7)	
Over AUD 104,000 per year	91 (41.6)	52 (37.7)	

Note: p-value computed from Independent samples *t*-Test or one-way ANOVA.

3.3. Findings of the regression analysis

To further explore the relationship between child and parental characteristics and normalised hair cortisol concentration, we conducted univariable linear regressions (Table 3). The significant associations between child's age, location (Western Australia), and family annual income with normalised hair cortisol concentration were preserved ($p < 0.03$ for all). However, by using univariable linear regression we unmasked several predictor relationships with normalised hair cortisol concentration that were not detected by assessing the difference between group means using t-tests. We found that autism diagnosis, BMI and the presence of co-occurring conditions, mother and father's age, and father's BMI, were significantly associated with normalised hair cortisol concentrations (Table 3). Unexpectedly autistic children had lower hair concentrations than non-autistic children ($p = 0.005$), children with co-occurring medical conditions had lower hair cortisol concentrations than those without ($p = 0.014$), and child's BMI, father's BMI, and maternal and paternal age were significantly inversely associated with hair cortisol concentration ($p < 0.02$ for all).

Findings of the multivariable regression model of the predictors that were statistically significant at the final step of the full model are shown in Table 4. Consistent with the descriptive statistics and univariable regression models, increase in child's age was significantly associated with lower hair cortisol concentration ($p \leq 0.016$), thus suggesting that children aged under 5 years had significantly higher normalised hair cortisol concentration than older children. We also found that an increase in family annual income was significantly associated with a decrease in normalised hair cortisol levels ($p = 0.001$). Recruitment site significantly affected normalised hair cortisol concentration, wherein compared to Victoria, the Queensland cohort had significantly higher levels of normalised hair cortisol levels ($p = 0.024$). The Western Australia and NSW sites also had higher cortisol levels, but the findings were non-significant. To further explore whether the recruitment site differences may be explained by differences in the distribution of family or child characteristics, we assessed interactions between site and the significant predictors from the univariable linear regression. No significant interactions between site and participant age, participant diagnosis, participant BMI, participant co-occurring condition, parental age, father's BMI, and family income were found. Finally, to explore if a shared household environment among autistic children and their non-autistic siblings influenced these findings, we repeated the

Table 2
Hair cortisol concentrations by child and parent-level characteristics.

Characteristics	N = 589	Hair cortisol concentration (pg/mg)	p-value
Site			
Western Australia	203	11.43 (22.36)	**0.050**
Victoria	85	6.37 (6.40)	
New South Wales	104	8.03 (11.27)	
Queensland	197	8.31 (12.22)	
Child-level characteristics			
Child's age			
Up to 5 years	216	12.52 (23.22)	**<0.001**
6-12 years	306	7.35 (8.82)	
13-17 years	67	5.67 (8.63)	
Gender			
Male	372	8.48 (12.32)	0.246
Female	217	10.05 (20.65)	
Participant type			
Autistic (includes ASD-undefined group)	307	8.50 (13.07)	0.377
Non-autistic (siblings and control group)	282	9.66 (18.51)	
Do they have a twin?			
No	481	8.95 (17.14)	0.832
Yes	33	8.31 (7.55)	
More than one autistic child in immediate family			
No	178	8.06 (10.32)	0.305
Yes	108	9.75 (17.52)	
Tanner stage			
Before puberty	129	6.55 (8.35)	0.130
During puberty	56	5.07 (4.72)	
Type of usual residence			
Parental home	505	7.89 (9.28)	0.376
Other residence	6	20.01 (30.58)	
Language spoken at home			
English	486	7.88 (9.58)	0.336
Other	22	9.92 (12.24)	
Other conditions			
No	408	9.83 (18.13)	0.077
Yes	181	7.32 (8.85)	
Maternal characteristics			
Mother's country of birth			
Australia	292	7.74 (8.95)	**0.045**
Other	297	10.35 (20.50)	
Mother's ethnicity			
Caucasian	311	7.83 (9.45)	0.518
Other	77	8.62 (9.70)	
Mother's education level			
High school level	104	8.03 (9.14)	0.846
Trade, certificate, other levels	118	8.32 (10.48)	
University degree	169	7.67 (8.95)	
Mother's employment status			
Home duties	78	8.37 (10.47)	0.595
Professional or skilled labour	305	7.74 (9.14)	
Mother's smoking status during pregnancy			
No	227	7.82 (10.87)	0.974
Yes	52	7.77 (9.16)	
Mother's drinking status during pregnancy			
No	81	8.08 (10.16)	0.799
Yes	199	7.72 (10.71)	
Paternal characteristics			
Father's country of birth			
Australia	272	8.09 (10.12)	0.168
Other	315	9.91 (19.59)	
Father's ethnicity			
Caucasian	317	7.82 (9.28)	0.413
Other	68	8.86 (10.54)	
Father's education level			
High school level	110	8.98 (11.66)	0.476
Trade, certificate, other levels	141	7.77 (8.17)	
University degree	128	7.56 (8.98)	
Father's employment status			
Professional	157	7.57 (8.81)	0.421
Skilled labour	198	8.40 (10.19)	
Father's smoking status during pregnancy			
No	195	7.44 (10.48)	0.304
Yes	64	9.04 (11.45)	

(*continued on next page*)

Table 2 (*continued*)

Characteristics	N = 589	Hair cortisol concentration (pg/mg)	p-value
Father's drinking status during pregnancy			
No	46	7.34 (8.42)	0.839
Yes	215	7.68 (10.63)	
Family annual income			
Up to AUD 70,000 per year	107	9.33 (9.72)	**0.005**
AUD 70,001 to 104,000 per year	107	8.52 (8.73)	
Over AUD 104,000 per year	143	6.09 (6.25)	

Note: p-values computed from Independent samples *t*-Test or one-way ANOVA.

multivariable regression model analysis with non-autistic siblings removed. No changes to the significant predictors of hair cortisol were found (Supplementary Table 1).

4. Discussion

To our knowledge, this is the largest study of hair cortisol concentration to be reported in autistic children. Additionally, this is the most comprehensive assessment of child, parental, and household/psychosocial characteristics in relation to hair cortisol concentrations in autistic children and non-autistic siblings and controls. Our findings suggest that chronic stress, as measured by hair cortisol concentration, does not differ between autistic children and non-autistic children when other individual attributes are taken into account. Such findings contradict the conclusions from some of the previous findings, including those by Ogawa et al. who reported hair cortisol level to be higher in 34 autistic children compared to 12 age-matched typically developing children (Ogawa et al., 2017). While this discrepancy may be attributable to differences in study protocols, statistical modelling, and participant characteristics, the current findings from a larger sample of children may indicate that chronic stress as indicated by hair cortisol concentration may be impacted by factors other than diagnosis such as age and psychosocial factors in autistic children. In this regard, our findings are supported by other studies assessing cortisol in saliva or blood of autistic children that have found no differences based on diagnosis (De Vaan et al., 2020) and at baseline prior to acute stress exposure (Spratt et al., 2012). Further, the current findings do not contradict previous research that has demonstrated that autistic children display enhanced acute cortisol reactivity to stress (Corbett et al., 2006; Spratt et al., 2012). Instead, the nature and type of events capable of inducing elevated blood or saliva cortisol or the magnitude and duration of their increases across a period of weeks or months may not be sufficient to translate to higher hair cortisol concentrations.

The findings from the multivariable regression model revealed several key individual characteristics – child's age, family income, and residential location (state), as significantly associated with hair cortisol concentration. Each of these factors might act as a stronger contributor to the variation in hair cortisol concentrations than the diagnosis of autism. Family income and residential site are known to be associated with HPA axis activation or modulation and represent lifestyle variables that are chronic in nature, supporting the contention that hair cortisol concentration effectively reflects persistent psychosocial and lifestyle stressors. Previous findings have demonstrated family income and residential characteristics to influence hair cortisol (Gray et al., 2018; Kunin-Batson et al., 2023; Rippe et al., 2016; Vaghri et al., 2013), especially in typically developing children. To our knowledge, this is the first time that psychosocial factors such as family income have been demonstrated to affect autistic children's hair cortisol levels, suggesting that poor socioeconomic background may be a potential mediator for chronic stress in autistic children just as it has been demonstrated in non-autistic children. For example, Vliegenthart et al.'s (2016) study found that neighbourhood level socioeconomic status based on neighbourhood measures of income, education and employment was significantly associated with hair cortisol adjusted for age and sex (Vliegenthart et al., 2016). In this regard, our findings that the children from the Queensland site significantly differed in hair cortisol concentration from the Victorian site (which had the lowest mean normalised hair cortisol concentration) deserve further exploration as to whether specific neighbourhood socioeconomic factors and sample ascertainment bias may explain this finding. Our own investigation into whether interaction of any of the significant predictors from the univariable linear regression (Table 3; participant age, participant diagnosis, participant BMI, participant co-occurring condition, parental age, father's BMI, and family income), yielded no significant location-based differences. However, we did not have access to residential postcode data that would allow more granular analysis to help identify if the distribution of participants between sites differed based on population density and environmental factors. In this regard, there is emerging evidence that residential areas such as urban upbringing could be associated greater cortisol excretion levels in response to acute stress (Steinheuser et al., 2014).

Our finding that family income was inversely associated with hair cortisol concentration is consistent with evidence of a positive association between socioeconomic status, of which income is a critical factor, and mental as well as physical health and wellbeing (Australian Institute of Health and Welfare, 2022). It is possible that family income impacts cortisol reactivity through stress responses associated with economic pressures and consequent household burden that should be further explored. Previous evidence also indicates similar conclusions that children from very low income households have higher hair cortisol accumulations (Kunin-Batson et al., 2023; Rippe et al., 2016; Vaghri et al., 2013). Taken together, these lines of evidence support that family income and its psychosocial consequences may have a unique impact on children's stress responses.

For the first time we show an age-associated change in hair cortisol concentration in a large sample of autistic children and demonstrate the same trajectory and association of hair cortisol with age as typically developing children. Hair cortisol concentration was significantly negatively associated with age. Children aged under 5 had the highest cortisol concentration that declined with increase in age. Reflective of 5 years being the mean age of diagnosis for ASD (van't Hof et al., 2021), the autistic group had a smaller proportion of participants under 5 years old and a higher proportion between 6 and 12 years than the non-autistic control group. Given that the univariable analyses indicated autistic participants to have lower cortisol than non-autistic participants, it is possible that participant type influenced the significant age-related association observed. However, the final multivariable regression model of the collapsed sample indicated participant age to be a better predictor of hair cortisol than participant type. Therefore, this finding may also reflect the impact of household stress and psychosocial adversity being most evident in preschool children, or even an age-related association with maternal cortisol levels. For, instance, Perry et al. (2022) found that younger children's hair cortisol concentration was more strongly associated with the mother's hair cortisol concentration than older children, which may explain the age-related decline in hair cortisol concentrations of children in our sample. Unfortunately, parental hair was not collected but future studies are encouraged to consider sampling parents in addition to their autistic children given the elevated burden, psychological distress and lower social support that has been reported among parents of autistic children (Picardi et al., 2018). This type of investigation may also help inform the family income-associated effects on child's hair cortisol concentration which is plausibly a stronger source of chronic stress for the parents.

Not all investigations of hair cortisol concentration and age in children are consistent with our findings. To our knowledge, the majority of studies report no association between age and hair cortisol concentration in children (Gerber et al., 2017; Rippe et al., 2016; Simmons et al., 2016) when recruiting within the 6–12 year age bracket. However, it is possible that had these studies recruited a sample of younger

Table 3
Univariable linear regression.

Characteristics	Coefficient (b) (95% CI)	SE	t	F	Adjusted R^2	p-value
Site						
Victoria	Reference					
New South Wales	0.039	0.145	0.272	3.065	0.0104	**0.028**
Queensland	0.166	0.129	1.292			
Western Australia	0.325*	0.128	2.530			
Child-level characteristics						
Child's age						
Up to 5 years	Reference					
6-12 years	−0.473***	0.086	−5.515	21.32	0.0646	**<0.001**
13-17 years	−0.716***	0.135	−5.299			
Gender						
Male	Reference					
Female	0.150	0.085	1.763	3.107	0.0036	0.079
Participant type						
Non-autistic (siblings and control group)	Reference					
Autistic	−0.226**	0.082	−2.759	7.614	0.0111	**0.005**
Do they have a twin?						
No	Reference					
Yes	0.106	0.181	0.587	0.344	−0.0013	0.558
More than one autistic child in immediate family						
No	Reference					
Yes	0.029	0.130	0.228	0.052	−0.0033	0.820
Tanner stage						
Before puberty	Reference					
During puberty	−0.073	0.154	−0.477	0.228	−0.0042	0.634
Type of usual residence						
Parental home	Reference					
Other residence	−0.085	0.401	−0.212	0.045	−0.0019	0.832
Language spoken at home						
English	Reference					
Other	0.296	0.212	1.399	1.957	0.0019	0.162
Other conditions						
No	Reference					
Yes	−0.220*	0.089	−2.470	6.102	0.0086	**0.014**
Child BMI	−0.031**	0.011	−2.834	8.030	0.0123	**0.005**
Total number of medications	−0.182	0.010	−1.832	3.355	0.0167	0.069
Maternal characteristics						
Mother's age	−0.023***	0.007	−3.523	12.41	0.0284	**0.001**
Mother's country of birth						
Australia	Reference					
Other	0.123	0.082	1.499	2.246	0.0021	0.135
Mother's education level						
High school level	Reference					
Trade, certificate, other levels	−0.066	0.131	−0.504	0.161	−0.0043	0.851
University degree	−0.061	0.122	−0.504			
Mother's employment status						
Home duties	Reference					
Professional or skilled labour	−0.040	0.123	−0.327	0.107	−0.0023	0.744
Mother's smoking status during pregnancy						
No	Reference					
Yes	−0.057	0.156	−0.367	0.135	−0.0031	0.714
Mother's drinking status during pregnancy						
No	Reference					
Yes	−0.082	0.133	−0.617	0.381	−0.0022	0.537
Mother's BMI	−0.005	0.008	−0.636	0.404	−0.0016	0.525
Paternal characteristics						
Father's age	−0.020***	0.006	−3.364	11.31	0.0267	**0.001**
Father's country of birth						
Australia	Reference					
Other	0.136	0.083	1.646	2.711	0.0029	0.100
Father's education level						
High school level	Reference					
Trade, certificate, other levels	−0.022	0.124	−0.176	0.084	−0.0049	0.920
University degree	−0.051	0.127	−0.405			
Father's smoking status during pregnancy						
No	Reference					
Yes	0.089	0.146	0.609	0.371	−0.0024	0.543
Father's drinking status during pregnancy						
No	Reference					
Yes	−0.028	0.163	−0.173	0.030	−0.0037	0.863
Father's BMI	−0.028*	0.012	−2.357	5.558	0.0151	**0.019**
Family annual income						
Up to AUD 70,000 per year	Reference					
AUD 70,001–104,000 per year	0.017	0.126	0.134	7.762	0.0366	**0.001**
Over AUD 104,000 per year	−0.383**	0.118	−3.256			

Note: *p-values computed from univariable linear regression modelling.* Signif. codes: ***p < 0.001; **p < 0.01; *p < 0.05.

Table 4
Multivariable linear regression model (Final step of the model).

Characteristics	Coefficient (b)	SE	t-value	p-value
Site				
Victoria	Reference			
New South Wales	0.118	0.194	0.604	0.546
Queensland	0.424	0.187	2.270	**0.024**
Western Australia	0.375	0.199	1.884	0.061
Child-level characteristics				
Child's age				
Up to 5 years	Reference			
6-12 years	−0.295	0.121	−2.429	**0.016**
13-17 years	−0.600	0.222	−2.700	**0.007**
Family annual income				
Up to AUD 70,000 per year	Reference			
AUD 70,001 to 104,000 per year	−0.044	0.135	−0.328	0.743
Over AUD 104,000 per year	−0.419	0.128	−3.281	**0.001**

Note: p-values computed from multivariable linear regression modelling. Variables with a p-value <0.25 from the univariable regression model were included in the final multivariable regression model.

participants, they may have unmasked an age-related effect. For instance, we found the most dramatic difference in hair cortisol concentration between children under 5 years to those between 5 and 12 years. There was a less pronounced decline in hair cortisol concentration in children over 12 years old. Consistent with our contention that a wider age distribution may be required to capture age-related hair cortisol level differences in children, a recent longitudinal study by Gunnar et al. (2022) found hair cortisol concentration significantly declined across early childhood and plateaued in middle childhood (Gunnar et al., 2022) and other studies report an age-related decline in hair cortisol concentration across children and teens (Perry et al., 2022; Wagner et al., 2019). Paradoxically, White and colleagues (2017) discovered a significant positive association between hair cortisol concentration and age in children younger than the age of five. However, the primary goal of this study was to investigate the impact of maltreatment on hair cortisol concentration in children which interacted with the age effect (White et al., 2017). One possible explanation is that in this instance of extreme traumatic circumstances, the impact may be different with older children being impacted more.

Therefore, a key strength of this study was our large sample size and consideration of demographic, social and phenotypic characteristics of our participants, which enabled a nuanced and holistic assessment of the relationship between hair cortisol concentration and autism. We objectively measured chronic stress by using hair cortisol concentration. Our analyses were robust, with blinded analysis of hair cortisol concentration, comprehensive statistical analysis, and consideration of potential influencing covariates. This is evidenced by several significant predictors of the univariable linear regressions that were surprisingly negatively associated with hair cortisol concentration. These include participant diagnosis, child BMI and the presence of co-occurring medical conditions, which have previously been reported to increase cortisol levels (Feller et al., 2014; Rippe et al., 2016; Vaghri et al., 2013). Given that these characteristics were not significant predictors in the multivariate regression model, these negative associations are likely to be artefacts of the significant age-related association and the higher cortisol levels occurring in children from families that are financially struggling discovered using the multivariable regression modelling. However, our study is limited by its cross-sectional design, meaning we were unable to demonstrate how family characteristics may dynamically influence hair cortisol concentration within individuals over time. Future studies are encouraged to adopt longitudinal experimental designs to unmask potential time-dependent relationships. It is also worth noting that the relationship between hair cortisol concentration and autism diagnosis, age, income and other demographic factors were measured categorically whereas analysis of these factors as continuous variables may yield further insights, especially in regard to the extent that individual autistic traits may be associated with chronic stress.

5. Conclusion

The current study, the largest of its kind involving autistic children, their siblings and non-autistic controls to date, suggests that hair cortisol concentration can be used as a potential biomarker of chronic stress and HPA axis modulation in response to psychosocial and lifestyle factors. Our findings offer promising insights into the experience of chronic stress in autistic children, suggesting that autism diagnosis plays a limited role in the variation in physiological stress responses compared with other individual factors. Assessment of parent hair cortisol concentrations in future studies may offer insights into the age, family and socioeconomic factors associated with hair cortisol concentration. Further research is warranted to ascertain if hair cortisol concentration can provide greater specificity and differentiation as a biomarker of autism characteristics or severity if used in conjunction with other biological parameters responsive to stress, such as microbiome or immune status, in addition to the psychosocial and anthropometric characteristics explored here.

Funding

This work is supported by the Neuroscience Mental Health and Addiction Clinical Academic Group of the Sydney Partnership for Health Education Research and Enterprise (SPHERE) funding to Valsamma Eapen, Anne Masi, Lin Kooi Ong and Adam K. Walker (Grants#: O200005). Adam K. Walker is supported by the Schizophrenia Research Institute and Neuroscience Research Australia (Grant#: GXX0049). Nisha E. Mathew is funded by the Australian Government Department of Education, Skills and Employment Research Training Program and a NeuRA top up award. Lin Kooi Ong is supported by the University of Southern Queensland Research Capacity Building Grant, the International Society for Neurochemistry (ISN) Career Development Grant, and Monash University Malaysia.

CRediT authorship contribution statement

Ping-I Lin: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **James Rufus John:** Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Anne Masi:** Conceptualization, Funding acquisition, Investigation, Methodology, Visualization, Writing – review & editing. **Lin Kooi Ong:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Nisha E. Mathew:** Data curation, Investigation, Methodology, Writing – review & editing. **Mohammed Ali Moni:** Data curation, Writing – review & editing. **Valsamma Eapen:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Adam K. Walker:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

None.

Acknowledgements

The authors would like to thank the children on the autism spectrum, their siblings and parents and other children who generously contributed their time and data/samples for the Australian Autism Biobank. The data used in this project were provided by Autism CRC with appropriate ethics approval. The Australian Autism Biobank is an initiative of Autism CRC established with funding received from the Australian Government and CRC partners. The authors acknowledge the Australian Autism Biobank Team at the various sites and their staff who supported the establishment of the Australian Autism Biobank: Telethon Kids Institute, University of NSW, La Trobe University, Mater Medical Research Institute, Institute for Molecular Biosciences: University of Queensland, Wesley Medical Research, University of Western Australia, Pathwest and Sydney Children's Hospital Network, Andrew Whitehouse, Dora Abbondanza, Gail Alvares, Erin Beattie, Jolene Berry, Vandhana Bharti, Grace Christou, Dominique Cleary, Paul A Dawson, Melanie De Jong, Cheryl Dissanayake, Kendra Dommisse, Valsamma Eapen, Mira Frenk, Jacob Gratten, Rachel Grove, Claire Hafekost, Maryam Haghiran, Alexis Harun, Nicole Hayes, Anjali Henders, Honey Heussler, Helen Holdsworth, Anneliese Hopkins, Anna Hunt, Rachel Jellett, Feroza Khan, Lauren Lawson, Deborah Lennon, Jodie Leslie, Anne Masi, Nisha Mathew, Tiana McLaren, Candice Michael, Melanie Muniandy, Melissa Neylan, Michaela Nothard, Brooke Peden, Mridu Radhakrishnan, Ola Rajapakse, Emma Raymond, Felicity Rose, Natalie Silove, Ashley Thomson, Leanne Wallace and Naomi Wray. The authors acknowledge the work in organising the hair samples and co-ordination of sample shipment by Adam J Lawther, Ph.D.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2024.05.052.

References

Alvares, G.A., Dawson, P.A., Dissanayake, C., Eapen, V., Gratten, J., Grove, R., Henders, A., Heussler, H., Lawson, L., Masi, A., 2018. Study protocol for the Australian autism biobank: an international resource to advance autism discovery research. BMC Pediatr. 18, 1–9.

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Pub.

Australian Institute of Health and Welfare, 2022. Health across Socioeconomic Groups.

Bryson, H.E., Goldfeld, S., Price, A.M.H., Mensah, F., 2019. Hair Cortisol as a Measure of the Stress Response to Social Adversity in Young Children, vol. 61, pp. 525–542.

Chung, S., Son, G.H., Kim, K., 2011. Circadian rhythm of adrenal glucocorticoid: its regulation and clinical implications. Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1812, 581–591.

Corbett, B.A., Mendoza, S., Abdullah, M., Wegelin, J.A., Levine, S., 2006. Cortisol circadian rhythms and response to stress in children with autism. Psychoneuroendocrinology 31, 59–68.

De Vaan, G., Beijers, R., Vervloed, M.P., Knoors, H., Bloeming-Wolbrink, K.A., De Weerth, C., Verhoeven, L., 2020. Associations between cortisol stress levels and autism symptoms in people with sensory and intellectual disabilities. Frontiers in Education. Frontiers Media SA, 540387.

Eapen, V., Islam, R., Azim, S.I., Masi, A., Klein, L., Karlov, L., 2022. Factors impacting parental quality of life in preschool children on the autism spectrum. J. Autism Dev. Disord. 1–12.

Faurholt-Jepsen, M., Frøkjær, V.G., Nasser, A., Jørgensen, N.R., Kessing, L.V., Vinberg, M., 2021. Associations between the cortisol awakening response and patient-evaluated stress and mood instability in patients with bipolar disorder: an exploratory study. Int. J. Behav. Dev. 9, 8.

Feller, S., Vigl, M., Bergmann, M.M., Boeing, H., Kirschbaum, C., Stalder, T., 2014. Predictors of hair cortisol concentrations in older adults. Psychoneuroendocrinology 39, 132–140.

Gerber, M., Endes, K., Brand, S., Herrmann, C., Colledge, F., Donath, L., Faude, O., Pühse, U., Hanssen, H., Zahner, L., 2017. In 6-to 8-year-old children, hair cortisol is associated with body mass index and somatic complaints, but not with stress, health-related quality of life, blood pressure, retinal vessel diameters, and cardiorespiratory fitness. Psychoneuroendocrinology 76, 1–10.

Gray, N., Dhana, A., Van Der Vyver, L., Van Wyk, J., Khumalo, N., Stein, D., 2018. Determinants of hair cortisol concentration in children: a systematic review. Psychoneuroendocrinology 87, 204–214.

Gunnar, M.R., Haapala, J., French, S.A., Sherwood, N.E., Seburg, E.M., Crain, A.L., Kunin-Batson, A.S., 2022. Race/ethnicity and age associations with hair cortisol concentrations among children studied longitudinally from early through middle childhood. Psychoneuroendocrinology 144, 105892.

Hadwin, J.A., Lee, E., Kumsta, R., Cortese, S., Kovshoff, H., 2019. Cortisol awakening response in children and adolescents with autism spectrum disorder: a systematic review and meta-analysis, 22, 118–124.

Hardiman, R.L., Bratt, A., 2016. Hypothalamic-pituitary-adrenal axis function in Fragile X Syndrome and its relationship to behaviour: a systematic review. Physiol. Behav. 167, 341–353.

Hus, V., Lord, C., 2014. The autism diagnostic observation schedule, module 4: revised algorithm and standardized severity scores. J. Autism Dev. Disord. 44, 1996–2012.

Kennedy, P.J., Cryan, J.F., Quigley, E.M.M., Dinan, T.G., Clarke, G., 2014. A sustained hypothalamic–pituitary–adrenal axis response to acute psychosocial stress in irritable bowel syndrome. Psychol. Med. 44, 3123–3134.

Kunin-Batson, A.S., Crain, A.L., Gunnar, M.R., Kelly, A.S., Kharbanda, E.O., Haapala, J., Seburg, E.M., Sherwood, N.E., French, S.A., 2023. Household income, cortisol, and obesity during early childhood: a prospective longitudinal study. J. Pediatr. 252, 76–82.

Lai, M.-C., Kassee, C., Besney, R., Bonato, S., Hull, L., Mandy, W., Szatmari, P., Ameis, S. H., 2019. Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis. Lancet Psychiatr. 6, 819–829.

Lydon, S., Healy, O., Reed, P., Mulhern, T., Hughes, B.M., Goodwin, M.S., 2016. A systematic review of physiological reactivity to stimuli in autism. Dev. Neurorehabil. 19, 335–355.

Maenner, M.J., Warren, Z., Williams, A.R., Amoakohene, E., Bakian, A.V., Bilder, D.A., Durkin, M.S., Fitzgerald, R.T., Furnier, S.M., Hughes, M.M., et al., 2023. Prevalence and characteristics of autism spectrum disorder among children aged 8 Years — autism and developmental disabilities monitoring network, 11 sites, United States, 2020. MMWR Surveillance Summaries 72, 1–14.

McCaw, Z.R., Lane, J.M., Saxena, R., Redline, S., Lin, X., 2020. Operating characteristics of the rank-based inverse normal transformation for quantitative trait analysis in genome-wide association studies. Biometrics 76, 1262–1272.

Ogawa, S., Lee, Y.-A., Yamaguchi, Y., Shibata, Y., Goto, Y., 2017. Associations of acute and chronic stress hormones with cognitive functions in autism spectrum disorder. Neuroscience 343, 229–239.

Perry, N.B., Donzella, B., Troy, M.F., Barnes, A.J., 2022. Mother and child hair cortisol during the COVID-19 pandemic: associations among physiological stress, pandemic-related behaviors, and child emotional-behavioral health. Psychoneuroendocrinology 137, 105656.

Picardi, A., Gigantesco, A., Tarolla, E., Stoppioni, V., Cerbo, R., Cremonte, M., Alessandri, G., Lega, I., Nardocci, F., 2018. Parental burden and its correlates in families of children with Autism Spectrum Disorder: A multicentre study with two comparison groups. Clin. Pract. Epidemiol. Ment. Health. 14, 143–176.

Ratto, A.B., Kenworthy, L., Yerys, B.E., Bascom, J., Wieckowski, A.T., White, S.W., Wallace, G.L., Pugliese, C., Schultz, R.T., Ollendick, T.H., 2018. What about the girls? Sex-based differences in autistic traits and adaptive skills. J. Autism Dev. Disord. 48, 1698–1711.

Rippe, R.C., Noppe, G., Windhorst, D.A., Tiemeier, H., van Rossum, E.F., Jaddoe, V.W., Verhulst, F.C., Bakermans-Kranenburg, M.J., van Ijzendoorn, M.H., van den Akker, E.L., 2016. Splitting hair for cortisol? Associations of socio-economic status, ethnicity, hair color, gender and other child characteristics with hair cortisol and cortisone. Psychoneuroendocrinology 66, 56–64.

Saxbe, D.E., Golan, O., Ostfeld-Etzion, S., Hirschler-Guttenberg, Y., Zagoory-Sharon, O., Feldman, R., 2017. HPA axis linkage in parent–child dyads: effects of parent sex. autism spectrum diagnosis, and dyadic relationship behavior. 59, 776–786.

Sharpley, C.F., Bitsika, V., Andronicos, N.M., Agnew, L.L., 2016. Further evidence of HPA-axis dysregulation and its correlation with depression in Autism Spectrum Disorders: data from girls. Physiol. Behav. 167, 110–117.

Simmons, J.G., Badcock, P.B., Whittle, S.L., Byrne, M.L., Mundy, L., Patton, G.C., Olsson, C.A., Allen, N.B., 2016. The lifetime experience of traumatic events is associated with hair cortisol concentrations in community-based children. Psychoneuroendocrinology 63, 276–281.

Spratt, E.G., Nicholas, J.S., Brady, K.T., Carpenter, L.A., Hatcher, C.R., Meekins, K.A., Furlanetto, R.W., Charles, J.M., 2012. Enhanced cortisol response to stress in children in autism. J. Autism Dev. Disord. 42, 75–81.

Stalder, T., Kirschbaum, C., 2012. Analysis of cortisol in hair–state of the art and future directions. Brain Behav. Immun. 26, 1019–1029.

Steinheuser, V., Ackermann, K., Schönfeld, P., Schwabe, L., 2014. Stress and the city: impact of urban upbringing on the (re) activity of the hypothalamus-pituitary-adrenal axis. Psychosom. Med. 76, 678–685.

Tager-Flusberg, H., Kasari, C., 2013. Minimally verbal school-aged children with autism spectrum disorder: the neglected end of the spectrum. Autism Res. : official journal of the International Society for Autism Research 6, 468–478.

Tye, C., Runicles, A.K., Whitehouse, A.J., Alvares, G.A., 2019. Characterizing the interplay between autism spectrum disorder and comorbid medical conditions: an integrative review. Front. Psychiatr. 9, 751.

Vaghri, Z., Guhn, M., Weinberg, J., Grunau, R.E., Yu, W., Hertzman, C., 2013. Hair cortisol reflects socio-economic factors and hair zinc in preschoolers. Psychoneuroendocrinology 38, 331–340.

van't Hof, M., Tisseur, C., van Berckelear-Onnes, I., van Nieuwenhuyzen, A., Daniels, A. M., Deen, M., Hoek, H.W., Ester, W.A., 2021. Age at autism spectrum disorder diagnosis: a systematic review and meta-analysis from 2012 to 2019. Autism 25, 862–873.

Vives, A.H., De Angel, V., Papadopoulos, A., Strawbridge, R., Wise, T., Young, A., Arnone, D., Cleare, A., 2015. The relationship between cortisol, stress and psychiatric illness: New insights using hair analysis. J. Psychiatr. Res. 70, 38–49.

Vliegenthart, J., Noppe, G., Van Rossum, E., Koper, J., Raat, H., Van den Akker, E., 2016. Socioeconomic status in children is associated with hair cortisol levels as a biological measure of chronic stress. Psychoneuroendocrinology 65, 9–14.
Wagner, M., Kratzsch, J., Vogel, M., Peschel, T., Gaudl, A., Ceglarek, U., Thiery, J., Hiemisch, A., Körner, A., Kiess, W., 2019. Hair cortisol concentration in healthy children and adolescents is related to puberty, age, gender, and body mass index. Horm. Res. Paediatr. 92, 237–244.
White, L.O., Ising, M., von Klitzing, K., Sierau, S., Michel, A., Klein, A.M., Andreas, A., Keil, J., Quintero, L., Müller-Myhsok, B., 2017. Reduced hair cortisol after maltreatment mediates externalizing symptoms in middle childhood and adolescence. JCPP (J. Child Psychol. Psychiatry) 58, 998–1007.