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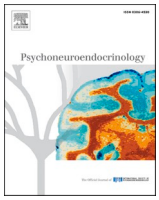

Exploring the genetic influence on hair cortisol concentration: Genetic association of rs11621961 on *SERPINA6/1* locus in the 2004 Pelotas Birth Cohort (Brazil)

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ABSTRACT

Genetics plays a critical role in regulating cortisol, as demonstrated by the association of the *SERPINA6/1* locus with plasma cortisol concentrations in a genome-wide association meta-analysis (GWAMA). These genes are integral to glucocorticoid transport and regulation, highlighting a direct genetic influence on cortisol availability. This study examines the genetic contribution to hair cortisol concentration (HCC) in adolescents from the 2004 Pelotas (Brazil) Birth Cohort at age 15, employing three distinct approaches: 1) polygenic score (PGS), 2) gene-based analysis, and 3) candidate variations analysis. A total of 1667 individuals were included. The cortisol-PGS was derived from the most recent morning plasma cortisol GWAMA study, and gene-based analyses were performed using MAGMA. For the analysis of candidate variants in the *SERPINA6/1* locus, we selected SNPs with P-values $\leq 5 \times 10^{-8}$ from the cortisol GWAMA and conducted *in silico* analyses to assess potential regulatory functions. Nineteen SNPs were tested. Our results revealed a significant association between rs11621961 and HCC after multiple testing correction. This intergenic SNP, located 1.1 kb from the 3′-untranslated region (UTR) of *SERPINA6*, showed that the T allele was associated with higher HCC (β=0.05, FDR-P = 0.038). Functional *in silico* analyses suggested that rs11621961 might influence gene expression and chromatin structure by altering motifs and acting as an expression quantitative trait locus (eQTL) in lymphoblastoid cell lines. However, neither the cortisol-PGS nor gene-based analyses showed an association with HCC. This study offers important contributions to the understanding of the genetic determinants of HCC, advancing the knowledge of the relationship between genetics and cortisol regulation in adolescents.

1. Introduction

Cortisol plays important roles in human physiology, with its primary function lying in the stress response (Iob and Steptoe, 2019). The hypothalamic-pituitary-adrenal (HPA) axis, encompassing the hypothalamus, pituitary gland, and adrenal cortex, orchestrates cortisol release in response to stress (Iob and Steptoe, 2019). Corticotropin-releasing hormone (CRH) and vasopressin (AVP) from the hypothalamus stimulate the pituitary gland to produce adrenocorticotropic hormone (ACTH), leading the adrenal gland to release cortisol and other glucocorticoids (Stephens and Wand, 2012). Cortisol is then transported by corticosteroid-binding globulin (CBG) and binds to cellular receptors. Chronic HPA axis dysregulation is linked to conditions like obesity (Ling et al., 2020), type 2 diabetes (Bawa et al., 2020),

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cardiovascular disease (Gan et al., 2022), depression and anxiety (Fiksdal et al., 2019), memory loss (Santoso et al., 2022) and other chronic diseases.

Cortisol circulates in plasma, either in free form or bound to transport proteins. Traditional measurement methods include blood, urine, and saliva, capturing cortisol levels at specific moments or over short periods (Turpeinen and Hämäläinen, 2013). Hair, however, offers a relatively novel approach by providing a measure of cumulative cortisol concentration, being explored in current studies as a marker of chronic stress and long-term HPA axis activity. Hair cortisol concentration (HCC) reflects the total cortisol secreted into hair over weeks or months, representing a longer-term hormonal profile (Iob and Steptoe, 2019). Free cortisol from follicular capillaries diffuses passively into the hair shaft during growth, with the cortisol in hair believed to mirror biologically active hormone levels (Russell et al., 2012) and correlate with systemic cortisol concentration (Greff et al., 2019). It is important to highlight that, although HCC is being explored as a biomarker of stress, it primarily reflects cumulative cortisol secretion over time and might be a marker of responses to perhaps ongoing stress (Colding-Jørgensen et al., 2023).

Despite undergoing important environmental modulation, genetics also influence circulating cortisol levels, with heritability estimated at 30–60 % (Mormede et al., 2011). For HCC heritability, Tucker-Drob and colleagues demonstrated that 65 % of the total variability of HCC was explained by additive genetic effects (Tucker-Drob et al., 2017). The genome-wide association meta-analysis (GWAMA) study for morning plasma cortisol of the CORtisol NETwork (CORNET) consortium, included 25,314 subjects and ~7 M SNPs, in 17 population-based cohorts of European ancestries and identified a single locus on chromosome 14 associated with morning plasma cortisol at genome-wide significance (Crawford et al., 2021). Using LD score regression, common SNP-heritability across the genome were found to explain 4.2 % of the phenotypic variation of morning plasma cortisol (Crawford et al., 2021). The *SERPINA6/1* locus identified in chromosome 14 includes two serine protease inhibitor genes (*SERPINA6* and *SERPINA1*), associated with cortisol concentration (Crawford et al., 2021). These genes encode proteins involved in glucocorticoid transport and regulation, emphasizing the direct genetic role in cortisol availability to tissues.

The first gene within this locus is *SERPINA6*, responsible for encoding the CBG protein. Cortisol is a fat-soluble molecule with low solubility in plasma, therefore, CBG binds to free cortisol and works with a transport protein (globulin), facilitating this circulation and constituting a circulating hormone reserve (Henley et al., 2016). Additionally, the *SERPINA1* gene, also situated in this locus, encodes the alpha-1-antitrypsin protein, an inhibitor of neutrophil elastase, which in turn cleaves CBG resulting in a decrease of CBG affinity for plasma cortisol (Nenke et al., 2016). Hence, genetic factors play a direct role in regulating cortisol availability in tissues. Limited studies, predominantly focusing on populations of European ancestry, and using plasma (Anderson et al., 2014; Bolton et al., 2014; Crawford et al., 2021) and saliva samples (Utge et al., 2018), have underscored the significance of the *SERPINA6/1* locus in cortisol concentrations.

This study aims to assess the genetic influence on HCC through three distinct approaches: 1) evaluate the impact of a morning plasma cortisol polygenic score (PGS) derived from Crawford et al. (2021) on HCC; 2) investigate the *SERPINA6/1* locus using gene-based analysis; and 3) examine the influence of candidate variations in the SERPINA6/1 locus on HCC in adolescent participants from the 2004 Pelotas (Brazil) Birth Cohort at age 15.

We hypothesize that genetic factors contribute to variability in HCC among adolescents. Specifically, we expect that: 1) the PGS will be significantly associated with HCC; 2) gene-based analysis will reveal a genetic influence of variations at the SERPINA6/1 locus on HCC; and 3) specific candidate variations within the *SERPINA6/1* locus will be associated with HCC levels in adolescents from the 2004 Pelotas (Brazil) Birth Cohort at age 15.

2. Material and methods

2.1. Study design and participants

The 2004 Pelotas Birth Cohort is a population-based birth cohort study in the city of Pelotas in southern Brazil. The cohort methodology has been described elsewhere (Santos et al., 2011, 2014; Tovo-Rodrigues et al., 2023). Between January 1st and December 31st, 2004, all children born in the city were identified, from which 4231 mothers gave informed consent and were included in the study with their children. All participants were re-assessed at ages 3 months (n = 3985; 95.7 % retention), 12 months (n = 3907; 94.3 %), 24 months (n = 3869; 91.4 %), 48 months (n = 3799; 89.8 %), 6 years (n = 3721; 86.7 %), 11 years (n = 3582; 84.3 %), 15 years (n = 1949; 48.5 %), and at 18 years (n = 3489; 85.0 %). The 15 years follow-up fieldwork was prematurely halted in March 2020 due to the Covid-19 pandemic. Therefore, the participants with genetic information and cortisol dosage data were included in the analyses. In the case of twins, the first child was included in the analyses in case both had genetic information or the one with genetic information.

2.2. Hair sample collection and cortisol quantification

Cortisol from the 2004 Pelotas cohort, at the 15-year follow-up, was obtained from hair samples collected by a trained field professional as detailed in Martins et al. (2023). The hair sample was collected from the posterior vertex of the scalp, a region commonly used in cortisol studies due to its consistent hair growth and reduced variability in cortisol deposition. After hair collection, a standardized protocol (Claire Buchan et al., 2021; Ferro and Gonzalez, 2020) was used for washing, grinding, extracting hormones, and measuring cortisol from hair in the laboratory. After extraction of hair cortisol, from the 3 centimeters of the hair cut closest to the scalp, samples were suspended in 150 μl of diluent for 24 hours and cortisol concentrations were measured in duplicate by ELISA technique using the Salivary Cortisol High Sensitivity Immunoassay Kit (Cat# 1–3002, Salimetrics, Pennsylvania), according to the manufacturer's instructions, using the ELISA plate reader (Spectramax 190). HCC were expressed in pg/mg. Outliers, corresponded to values 4 standard deviations (SD) away from the HCC mean using raw data, were eliminated from the analytical sample. Ten values were eliminated. After that, due to the right-tailed nature of the distribution, the HCC values were log transformed for inclusion in the regression models (Variance = 0.19; Skewness = 0. 01; Kurtosis = 3.67). The histogram of log transformed distribution of HCC is presented in Supplementary Figure 1.

2.3. Genomic data

The saliva samples for DNA analysis were collected at 6 years of age using the Oragene Genotek® – 250 DNA collection kit as described elsewhere (Santos et al., 2014), which contained a stabilizing buffer to preserve DNA integrity. Following DNA extraction, the DNA samples were stored at −80°C for long-term preservation. This storage condition ensured that the DNA remained stable and intact for subsequent analysis. Genomic DNA was obtained following the manufacturer's instructions. DNA was quantified by spectrophotometry using the NanoDrop equipment (Thermo Scientific™). DNA samples were genotyped using the Infinium Global Screening Array 2 (Illumina). To carry out quality control of SNPs and samples we used the PLINK software v.1.9 (Chang et al., 2015), which allowed filtering in 6 steps: 1) removal of markers and individuals with a 2 % or more cut of missing data; 2) sex inconsistency, check based on the estimated value of F: men > 0.8 and women < 0.2; 3) removal of markers from non-autosomal chromosomes and removal of markers with a less frequent allele frequency (MAF) below 1 %.; 4) removal of markers that were not in Hardy-Weinberg Equilibrium (HWE) with P-value $< 1 \times 10^{-6}$; 5) removal of individuals

with high or low heterozygosity rate (standard deviation +/- 3); 6) removal of individuals with a high degree of kinship (> 0.2).

After filtering the genotyped dataset according to quality control standards, imputation of the remaining non-genotyped variants was performed based on general population data from the 1000 Genomes Project (phase 3) as a reference panel (1000 Genomes Project Consortium, 2012), using SHAPEIT 2 (O'Connell et al., 2014) and MINIMAC3 (Delaneau et al., 2013). After imputation, autosomal variants were filtered to include only those with: < 2 % missing genotypes, minor allele frequency ≥ 0.01, imputation quality (r^2) > 0.3, and a HWE deviation with *P*-value $> 1 \times 10^{-6}$. After the application of the quality control filters, 11,811,746 variants were retained.

2.4. Cortisol polygenic score

The cortisol-PGS was created based on the summary statistics of the most recent GWAMA of morning plasma cortisol by the CORNET consortium (Crawford et al., 2021). The CORNET consortium extended its GWAMA to 25,314 subjects, in 17 population-based cohorts of European ancestry. The information available in the summary statistics of the study was retrieved, and quality control was performed, with imputation quality score (INFO) ≥ 0.8 and least frequent allele frequency (MAF) ≥ 0.01. The major histocompatibility complex (MHC) region (chr6: 26–33 M) and sexual chromosomes were removed.

The PGS was created using PRS-CS and PLINK1.9 softwares. PRS-CS is a Bayesian approach that utilizes GWAS summary statistics and linkage disequilibrium (LD) data to estimate the effect sizes of individual variants on a given trait (Ge et al., 2019). By applying a continuous shrinkage prior to SNP effect sizes, the method minimizes the impact of noisy SNPs, thereby enhancing the precision of PGS. In this analysis, we used the default parameter settings and incorporated an LD reference panel derived from European samples in the 1000 Genomes Project, which aligns with the ancestry of the GWAS summary statistics. The scores were exported and analyzed in *Stata* 17 (StataCorp, 2021). The R^2 plot was generated using the R programming language.

2.5. Gene-based association analysis

We examined the association at the gene level. The *SERPINA*6 and *SERPINA1* genes were tested individually. Analysis was performed using MAGMA software (version 1.06) (de Leeuw et al., 2015) and an annotation window of 10 kb window upstream and downstream of each gene was tested.

2.6. SERPINA6/1 SNPs

The GWAMA study for morning plasma cortisol identified a single locus on chromosome 14 associated with morning plasma cortisol at genome-wide significance. This locus comprises the serine protease inhibitor genes *SERPINA6* and *SERPINA1* (*SERPINA6/1* locus) (Crawford et al., 2021). For the analysis of candidate *SERPINA6/1* locus SNPs, we selected the SNPs with *P*-values $\leq 5 \times 10^{-8}$ from the GWAMA study, as these represent genome-wide significant associations for the phenotype of interest. We retrieved a total of 19 SNPs that were also present both in the GWAMA study summary statistics and in the 2004 Pelotas Cohort dataset (genotyped or imputed). Supplementary Table 1 presents these SNPs with the GWAMA description and with the parameters of the 2004 Pelotas Cohort.

2.7. Covariates

Sample characteristics were characterized according to variables collected at birth, encompassing child sex (assigned at birth: male or female), child skin color (identified by the mother and classified as black Brazilians, white Brazilians or others, following the criteria of the Brazilian Institute of Geography and Statistics), child birth weight (continuous, in grams), child prematurity (categorized as gestational age <37 weeks, no and yes), family income (monthly income in Reais, distributed in terciles with the first tercile representing the poorest and the third tercile representing the richest group), and maternal schooling (years of formal education completed, categorized as 0–4, 5–8 or ≥9 years). Child sex and the first 10 principal components (PCs) of ancestry served as covariates for adjusting the genetic analysis, with PC analysis conducted using PLINK1.9 (Chang et al., 2015) based on the entire genomic dataset.

In sensitivity analyses, hair characteristics were included as covariates to assess potential confounding effects on the association. At the 15-year follow-up, HCC and hair characteristics such as type of hair (straight, wavy, curly, coily), natural hair color (brown, black, blond, gray, or red), frequency of hair wash per week (categorized as less than 3 times, 4–6 times, or more than 7 times), season of hair collection (spring or summer – south hemisphere), region of the head for hair collection (vertex or other parts of the scalp) and corticosteroid use in the last 3 months (yes or no) were evaluated. Participants provided responses for hair characteristics, while the medication variable was answered by the participant's mother or guardian. Descriptive statistics for HCC, as it was not normally distributed, are presented as medians and interquartile ranges.

2.8. Statistical analysis

We initially conducted a descriptive analysis of the sample, comparing adolescents included in the current study with those from the entire cohort according to covariables using Pearson's chi-square tests and Analysis of Variance (ANOVA). The cortisol-PGS score was exported to *Stata* 17 (StataCorp, 2021) and the association with HCC at age 15 was analyzed through linear regression. Percentage variance explained by the PGS for HCC was calculated as the difference between the two R^2 (ΔR^2) aiming to assess the PGS's predictive ability.

The candidate *SERPINA6/1* locus SNPs were tested for association with HCC at age 15, through independent linear regression analysis in PLINK v1.9 (Chang et al., 2015), using the additive genetic model. The regression models were adjusted for sex and the first 10 principal components (PCs) of ancestry. All results were presented as beta coefficients (β), with 95 % confidence interval (95 % CI) and *P*-values. The alpha for statistical significance was 0.05. Afterwards, Benjamini-Hochberg correction (FDR – false discovery rate) was performed for multiple tests to eliminate spurious results.

Linkage disequilibrium (LD) pattern plots of the selected SNPs were generated by Haploview 4.2 (Barrett et al., 2005). Both D' and r^2 LD metrics were used to visualize the SNP pairwise LD plots. The LD block was estimated though the confidence interval (CI) method by Gabriel and collaborators (2002).

For the gene-based association test, we used the Bonferroni-adjusted significance threshold of p = 0.025 (regarding 2 tests/genes).

2.9. Sensitivity analysis

To evaluate the potential confounding effects of various hair characteristics on the association, we conducted sensitivity analyses. The first analysis involved incorporating key hair-related variables into the linear regression model, specifically: natural hair color, hair type, hair washing frequency, season of hair collection and region of the head for hair collection (Model 1). To address the potential bias of nonspecific quantification in the ELISA experiment, we further refined our sample to include only participants who reported not having used any corticosteroids in the past three months prior to the hair collection (Model 2). This adjustment was made in addition to the variables in Model 1. Both models were adjusted for the first 10 PCs and sex. To control for false positives due to multiple testing, we applied the Benjamini-Hochberg FDR test.

Moreover, to account for the heterogeneous ancestry of our sample,

we conducted a sensitivity analysis by stratifying the dataset. Specifically, we performed a separate analysis on the largest ancestry group, White Brazilians, after filtering out principal component analysis (PCA) outliers within this group. This approach aimed to assess the robustness of our findings while minimizing potential confounding effects related to population structure.

2.10. In Silico functionality analysis

To provide further information about the putative regulatory function of each candidate *SERPINA6/1* locus marker included in this study, we assessed Regulome DB v2.1 (Dong et al., 2023), Combined Annotation Dependent Depletion (CADD) (Rentzsch et al., 2021) and HaploReg v4.2 – Broad Institute (Ward and Kellis, 2012) tools. RegulomeDB v2.1 is a database that annotates SNPs with known and predicted regulatory elements in the intergenic regions of the *H. sapiens* genome. The RegulomeDB v2.1 rank scoring scheme refers to the supporting evidence for a particular location or variant, if more supporting data is available, the higher is its likelihood of being functional and hence receives a higher score (with 1 being higher and 7 being lower score). The RegulomeDB v2.1 probability score is ranging from 0 to 1, with 1 being most likely to be a regulatory variant. There is an overall positive correlation between the ranking scores and the probability scores (Dong et al., 2023). CADD is a tool for scoring the deleteriousness of single nucleotide variants and insertion/deletion variants in the human genome. CADD integrates multiple annotations into one metric by contrasting variants that survived natural selection with simulated mutations (Rentzsch et al., 2021). CADD indicates if a variant is declared 'pathogenic' (or 'functional' or 'deleterious') as opposed to 'benign' (or 'non-functional' or 'neutral') across all datasets. A CADD PHRED-score is a log10-derived rank score that ranges from 1 to 99, been scaled so that each point represents a 10-fold increase in rank. For instance, a score of 10 means the variant is among the top 10 % most deleterious variants, a score of 20 places the variant in the top 1 %. Higher CADD PHRED scores suggest a greater likelihood that a variant is deleterious or functionally significant, and scores typically above 20 are considered indicative of highly deleterious variants that are likely to affect biological function. HaploReg v4.2 is a tool for exploring annotations of the noncoding genome in variants. Information on the chromatin state and protein binding annotation from the Roadmap Epigenomics and The Encyclopedia of DNA Elements (ENCODE) projects are functional evidence integrated in this dataset (Ward and Kellis, 2012).

2.11. Ethical standards

The research protocol of the 2004 Pelotas Birth Cohort 15 years follow up was approved by the Research Ethics Committee of the Faculty of Medicine at the Federal University of Pelotas and by the Ethics Committee for the Analysis of Research Projects of the University of São Paulo under the number of approvals 40602124 and 889,753. The Brazilian National Commission for Research Ethics also approved the use of genomic data. Written informed consent was obtained from the mothers or legal guardians, and adolescents also signed an informed consent form.

3. Results

3.1. Sample characteristics

Descriptive statistics for the sample analyzed in this study are presented in Table 1. HCC data were obtained during the 15-year follow-up of the 2004 Pelotas Birth Cohort, while genetic information was derived from saliva DNA samples collected at age 6, with a genotyped sample size of 3472 individuals. HCC measurements were available for 1826 participants at the 15-year follow-up. For the present analysis, we included individuals with both genetic data and HCC measurements,

Table 1
Characterization of the sample according to socioeconomic, demographic and biological covariates assessed during the perinatal period, and comparison between participants included in the analyses and the entire cohort. 2004 Pelotas Birth Cohort study, Brazil.

Variables	Included in the study (n = 1667)		Entire cohort (n = 4231)		*P*-value
	N	%(95 %CI)	N	%(95 %CI)	
Perinatal follow up					
Sex					0.057
Male	819	49.1 (46.7;51.5)	2195	51.9 (50.4;53.4)	
Female	848	50.9 (48.5;53.3)	2036	48.1 (46.6;49.6)	
Skin color*					0.396
White brazilians	1167	70.0 (67.7;72.2)	2726	68.1 (66.7;69.6)	
Black brazilians	192	11.5 (10.1;13.1)	482	12.1 (11.1;13.1)	
Others	308	18.5 (16.7;20.4)	790	19.8 (18.5;21.0)	
Birth weight*					**< 0.001**
Mean (SD)	1667	3197 (505.1)	4226	3150 (566.2)	
Child prematurity*					**0.008**
No	1468	88.1 (86.5;89.6)	3603	85.5 (84.4;86.5)	
Yes	198	11.9 (10.4;13.5)	612	14.5 (13.5;15.6)	
Family income (in terciles)					**< 0.001**
1st (poorest)	384	23.1 (21.1;25.1)	1416	33.5 (32.1;35.0)	
2nd	614	36.8 (34.5;39.2)	1406	33.2 (32.0;34.7)	
3rd (richest)	669	40.1 (37.8;42.5)	1409	33.3 (31.9;34.7)	
Maternal schooling (in years)*					0.117
0–4	232	14.0 (12.4;15.8)	654	15.6 (14.5;16.7)	
5–8	666	40.3 (37.9;42.6)	1731	41.3 (39.9;42.8)	
9 +	756	45.7 (43.3;48.1)	1801	43.1 (41.5;44.5)	
15-year follow up					
HCC (pg/mg)*					
Median (IQR)	1667	3.67 (2.81;4.87)	-	-	
Hair type*					
Straight	619	37.2 (34.9;39.5)	-	-	
Wavy	540	32.4 (30.2;34.7)	-	-	
Curly	318	19.1 (17.3;21.1)	-	-	
Coily	188	11.3 (9.8;12.9)	-	-	
Natural hair color*					
Brown	1025	61.6 (59.2;63.9)	-	-	
Black	535	32.1 (30.0;34.4)	-	-	
Blonde	95	5.7 (4.7;6.9)	-	-	
Red	10	0.6 (0.3;1.1)	-	-	
Hair washing frequency (per week)*					
≤ 3 times	376	22.6 (20.6;24.6)	-	-	
4–6 times	414	24.9 (22.8;27.0)	-	-	
≥ 7 times	875	52.5 (50.1;55.0)	-	-	

95 % CI: confidence interval of 95 %. SD: standard deviation. *missing values. Bold caption denotes significance.

resulting in a final sample of 1667 participants. The reduced sample size compared to the original cohort reflects the timing of DNA collection and hair collection/HCC measurement. DNA collection occurred at age 6, when follow-up rates were higher. However, data collection during the 15-year follow-up was prematurely interrupted in March 2020 due to the COVID-19 pandemic, limiting the availability of HCC available information.

Approximately half of the participants were females (50.9 %), and most of their mothers declared their children White Brazilians (70.0 %). The mean birth weight was 3179 g (standard deviation [SD] ± 505.1 g). Exactly 11.9 % were born prematurely. Considering income and educational levels, 40.1 % of participants were from the highest family income tercile of the baseline population, and nearly half (45.7 %) had mothers with 9 or more years of schooling. Regarding hair characteristics, the majority had brown hair (61.6 %), about one-third (37.2 %) had straight hair, and over half (52.5 %) reported washing their hair seven times or more per week. Only a small fraction (11.4 %) had used corticosteroid medications in the three months preceding hair collection. The observed median hair cortisol among participants was 3.67 pg/mg (IQR: 2.81;4.87). Adolescents in the analytic sample exhibited higher average birth weight and lower prematurity prevalence and were also more likely to come from the wealthiest income tercile, compared to the entire cohort (n = 4321, Table 1).

3.2. Cortisol-PGS prediction for HCC

We did not find statistically significant results between the cortisol-PGS and HCC at 15 years (β = −0.004, 95 % CI = −0.025; 0.017, P = 0.729; ΔR^2 = 0.00007 %); Table 2 and Supplementary Figure 2). After sensitivity analyses, results remained consistent regarding the direction and magnitude of associations, showing no statistically significant associations between PGS and HCC (Supplementary Table 2). We also conducted a sensitivity analysis by stratifying the dataset and analyzing White Brazilians, where the results remained consistent regarding the direction and magnitude of associations, showing no statistical significance (Supplementary Table 3).

3.3. Gene-based association analysis

No significant associations were identified for the *SERPINA6* and *SERPINA1* genes within the tested annotation window (*SERPINA6* P = 0.274; *SERPINA1* P = 0.252), based on the Bonferroni-adjusted significance threshold. Sensitivity analysis by stratifying the dataset and analyzing White Brazilians also did not identified significance. Detailed results are provided in Supplementary Table 4 and 5.

3.4. Association between SERPINA6/1 variants and HCC

The results for regression analysis exploring the association between SNPs at the *SERPINA6/1* locus and HCC, adjusted for 10 first PCs and sex are presented in Table 3. Significant associations were observed for rs11621961, rs2749530, rs2749529, rs3762132, and rs1243171 with HCC, prior to the application of corrections for multiple testing. Following the correction for multiple tests, only the association of rs11621961 with HCC remained statistically significant (Table 3).

The T allele of rs11621961, an intergenic SNP located approximately 1.1 kb from the 3′-untranslated region (UTR) of SERPINA6, was associated with higher HCC (β=0.05, 95 % CI=0.02; 0.08, P = 0.002, FDR-P = 0.038). Before correction for multiple tests, the G alleles of rs2749530 and rs3762132 were associated with lower HCC [(β=-0.04, *P* = 0.018, FDR-*P* = 0.068) and (β=-0.04, *P* = 0.014, FDR-*P* = 0.068), respectively]. Similarly, the A alleles of the intergenic SNPs rs2749529 and rs1243171 were also associated with lower HCC [(β=-0.04, *P* = 0.016, FDR-*P* = 0.068) and (β=-0.04, *P* = 0.009, FDR-*P* = 0.068), respectively (Table 3)].

The sensitivity analyses yielded consistent results. In Model 1, adjusted for hair characteristics besides 10 PCs and sex, the same five SNPs were associated with HCC prior to correction for multiple tests, with only rs11621961 remaining statistically significant afterwards. In Model 2, which accounted for the removal of those who had corticosteroid use, eight SNPs, including rs11621961, were significant before applying the FDR correction. Post-correction, however, only maintained statistical significance for rs11621961 (Supplementary Table 6). Despite small differences in p values, the direction and magnitude of associations were similar across both the main analysis and the sensitivity analysis models (Table 3, Supplementary Table 6).We also conducted a sensitivity analysis by stratifying the dataset and analyzing White Brazilians. While this subgroup analysis had limited statistical power to detect associations, the direction of the observed associations remained consistent with those found in the overall sample. Results can be found in Supplementary Table 7.

LD analysis of the 19 *SERPINA6/1* locus SNPs identified two haplotype blocks that ranged in size from 6 kb to 20 kb (Fig. 1). Four variants that were associated with HCC prior to correction for multiple tests (rs2749530, rs2749529, rs3762132, and rs1243171) are placed in block 2 and present strong LD between them (D′ ranging 0.96–0.98 and r^2 ranging from 0.70 to 0.99). The only remained associated variant rs11621961, however, is outside de blocks and present lower LD with the remaining variants (D′ranging from 0.63 to 0.72 and r^2 ranging from 0.5 to 0.24).

Table 2
Results of linear regression examining the association of cortisol polygenic score (cortisol-PGS) with hair cortisol concentration (HCC) at age 15. 2004 Pelotas Birth Cohort study, Brazil (n = 1667).

	Cortisol-PGS				
	β_{crude}***(95 %CI)***	***P-value***	$B_{adjusted}$***(95 %CI)***	***P-value***	ΔR^2
HCC ln (pg/mg)	−0.001 (−0.023;0.020)	0.729	−0.004 (−0.025;0.017)	0.729	0.00007

HCC: hair cortisol concentration; PGS: Polygenic score; β: beta coefficient; 95 % CI: 95 % confidence interval.
Adjusted by sex and 10 principal components of ancestry.

3.5. In silico functionality analysis and exploration of SNP regulatory functions

In silico analyses were undertaken to explore the regulatory potential of the examined variants. According to RegulomeDB, rs11621961 was ranked as 1 f, signifying a high likelihood of influencing gene expression and/or chromatin structure (eQT/caQTL + TF binding/chromatin accessibility peak). It boasts a probability score of 0.55 and a low CADD score of 2.448 (see Table 4). HaploReg data, as presented in both Table 4 and Supplementary Table 8, suggests that rs11621961 modifies the SMAD motif and serves as an expression quantitative trait locus (eQTL) for *IFI27L1* in lymphoblastoid cell lines.

The majority of the SNPs similarly exhibit a RegulomeDB rank score of 1 f, indicating moderate to high regulatory potential, accompanied by low CADD scores (Table 4). Other variants at the *SERPINA6/1* loci are situated in regions marked as promoters and enhancers in various tissues, including liver, blood, skin, breast, and gastrointestinal tissues. These variants impact several motifs and predominantly act as eQTLs for *SERPINA1* in whole blood and lymphoblastoid cell lines (see Table 4, Supplementary Table 8).

4. Discussion

In this study, we assessed the genetic component of HCC, investigating its polygenicity and the role of candidate SNPs at the *SERPINA6/1* locus in HCC among adolescents in a population-based Brazilian cohort.

Table 3
Results of adjusted linear regression model investigating the association between candidate SNPs at the *SERPINA6/1* locus and hair cortisol concentration (HCC) at age 15. 2004 Pelotas Birth Cohort Study, Brazil. (n = 1667).

		Hair Cortisol Concentration ln (pg/mg)						
SNP	***Gene Location***	***Position (GRCh37)***	***EA***	***RA***	***Info Score***	$\beta_{adjusted}$ ***(95 %CI)***	***P-value***	***P-value after FDR correction****
rs11621961	**1 kb 3' of *SERPINA6***	94,769,476	**T**	**C**	0.99647	**0.05 (0.02;0.08)**	**0.002**	**0.038**
rs11629171	Intron (*SERPINA6*)	94,773,450	T	C	0.99938	−0.02 (−0.06;0.01)	0.147	0.207
rs7161521	Intron (*SERPINA6*)	94,787,288	T	C	0.86666	−0.01 (−0.05;0.03)	0.656	0.710
rs941599	Intron (*SERPINA6*)	94,788,341	T	C	0.86597	−0.01 (−0.05;0.03)	0.656	0.710
rs2281518	Intron (*SERPINA6*)	94,789,117	G	A	0.86549	−0.01 (−0.05; 0.03)	0.710	0.710
rs12589136	4 kb 5' of *SERPINA6*	94,793,686	T	G	0.86634	−0.01 (−0.05; 0.03)	0.683	0.710
rs1956174	26 kb 5' of *SERPINA6*	94,816,121	A	G	0.92827	−0.03 (−0.06;0.01)	0.123	0.207
rs2749530	**27 kb 5' of *SERPINA6***	**94,816,299**	**G**	**A**	0.93103	**−0.04 (−0.07;−0.01)**	**0.018**	0.068
rs2749529	**23 kb 3' of *SERPINA1***	**94,820,459**	**A**	**T**	0.92538	**−0.04 (−0.07; −0.01)**	**0.016**	0.068
rs4905188	22 kb 3' of *SERPINA1*	94,821,237	C	T	0.98718	−0.03 (−0.06; 0.01)	0.164	0.207
rs2749527	16 kb 3' of *SERPINA1*	94,827,068	T	C	0.90816	0.02 (−0.01; 0.05)	0.118	0.207
rs12588394	13 kb 3' of *SERPINA1*	94,829,668	T	G	0.92346	−0.03 (−0.07; 0.01)	0.105	0.207
rs3819333	13 kb 3' of *SERPINA1*	94,830,555	T	C	0.93225	−0.03 (−0.06; 0.01)	0.152	0.207
rs941595	11 kb 3' of *SERPINA1*	94,832,016	C	T	0.91781	−0.03 (−0.07; 0.01)	0.081	0.192
rs1950652	8.7 kb 3' of *SERPINA1*	94,834,336	A	G	0.88493	−0.03 (−0.07; 0.00)	0.062	0.190
rs3762132	**8.5 kb 3' of *SERPINA1***	**94,834,575**	**G**	**A**	0.88772	**−0.04 (−0.07; −0.01)**	**0.014**	0.068
rs3762130	8.2 kb 3' of *SERPINA1*	94,834,861	G	C	0.89742	−0.03 (−0.07; 0.00)	0.070	0.190
rs3748319	7.4 kb 3' of *SERPINA1*	94,835,647	T	C	0.89933	−0.03 (−0.06; 0.01)	0.156	0.207
rs1243171	**6.3 kb 3' of *SERPINA1***	**94,836,784**	**A**	**G**	0.88126	**−0.04 (−0.07; −0.01)**	**0.009**	0.068

SNP: single nucleotide polymorphism; EA: effect allele; RA: reference allele; β: Beta coefficient; 95 %CI: 95 % confidence interval. Adjusted for sex and the 10 principal components of ancestry.*Benjamini-Hochberg Adjusted P-value. Bold caption denotes significance prior or after FDR correction.

Our results suggest that rs11621961, located in *SERPINA6* gene, has a significant effect on HCC. No significant associations were observed between cortisol PGS and HCC. Similarly, the gene-based analyses did not yield any statistically significant results. Conclusions were strengthened through various sensitivity analyses, which were consistent with these findings.

This study provides new data on potential genetic factors influencing HCC, a marker of cumulative cortisol exposure. Prior genetic studies have predominantly focused on acute cortisol levels, utilizing blood (Anderson et al., 2014; Bolton et al., 2014; Crawford et al., 2021) and saliva (Utge et al., 2018) for analysis. Our findings suggest a genetic mechanism that affects cumulative cortisol levels. We found a significant association between the rs11621961 SNP and HCC. Other SNPs in the same region (rs2749530, rs2749529, rs3762132, and rs1243171) showed initial associations, but these did not remain significant after adjusting for multiple comparisons. However, their potential role in HCC cannot be disregarded, especially considering our sample size, which is moderate to detect such associations. The observed association of rs11621961 in the *SERPINA6/1* locus aligns with recent GWAMA findings regarding the locus's significant role in regulating morning plasma cortisol concentration (Crawford et al., 2021). This finding implies that similar genetic factors may affect cortisol transport and availability in both plasma and hair, indicating a potential overlap in the underlying genetic mechanisms. It is also important to emphasize that, while cortisol is being explored as a biomarker of stress, different measurement methods capture distinct aspects of cortisol activity within the body. Hair cortisol might serve as a marker of accumulated or integrated cortisol levels for, maybe, ongoing stress (Colding-Jørgensen et al., 2023), whereas GWAS utilized in this study is based on morning plasma cortisol, which reflects basal secretion under typical physiological conditions. Also important, results about HCC relation with stress-related mental disorders like depression or anxiety disorders are still inconsistent (Malisiova et al., 2021).

However, regarding the specific role of the rs11621961 SNP, our study reports an effect direction that contrasts with previous GWAS findings (Bolton et al., 2014; Crawford et al., 2021) identifying the T allele as associated with lower morning plasma cortisol concentrations. In contrast, our results indicate a positive association between the T allele and higher HCC. This association remained consistent even after adjusting for potential confounders, including genomic ancestry, sex, and corticosteroid use and hair characteristics. Importantly, replication tests conducted in three independent cohorts included in the meta-analysis, as well as a subsequent meta-analysis focusing on morning plasma cortisol levels, identified higher cortisol concentrations associated with the C allele (Bolton et al., 2014).

This discrepancy might also be attributed to variations in allele frequencies and LD blocks across different ethnic groups (Wall and Pritchard, 2003). SNPs identified in GWAS are predominantly from populations of European descent. When replicated in diverse populations, a 'flip-flop' phenomenon can occur, whereby a variant may act as a risk factor in one population but prove protective in another, impacting the efficacy of risk prediction models. This phenomenon has been recognized as a challenge in drawing causal inferences in gene-disease association replication studies (Lin et al., 2007). Another explanation could be due to a difference in regulation between acute and chronic conditions, since previous works analyzed cortisol from plasma and saliva (an acute measure of stress) and our study analyzed cortisol from hair (a measure of cumulative cortisol concentration). Consequently, the association's directional discrepancy underscores the need for further comprehensive research to elucidate the underlying mechanisms and implications of this genetic variation on cortisol regulation.

Our PGS findings align with cortisol GWAMA results (Crawford et al., 2021), highlighting the predominant role of the *SERPINA6/1* locus in cortisol variability and a less pronounced effect from other additive genetic markers. Notably, research demonstrating polygenic effects on saliva and hair cortisol has primarily focused on GWAS of psychiatric disorders, mental health, substance abuse and obesity (Ahrens et al., 2022; Taylor et al., 2022; Marceau et al., 2022; Sun et al., 2018). The study from Rietschel and colleagues (2017) explored the heritability of HCC using formal genetic twin models and evaluated the impact of a PGS on HCC in adolescent and young adult twins from the Brisbane Longitudinal Twin Study. The cohort consisted of 116 monozygotic and 201 dizygotic twin pairs. The PGS was derived from summary statistics of the Bolton GWAS on plasma cortisol conducted by the CORNET Consortium, which included data from 12,597 individuals. Twin analyses revealed a high heritability for HCC (72 %); however, the PGS did not account for any variance in HCC, aligning with our results (Rietschel et al., 2017).

The GWAS conducted by Bolton et al. (2014), in a sample size of 12, 597, reported that approximately 1 % of the variance in plasma cortisol can be attributed to genetic variations within the *SERPINA6/1* region on chromosome 14. Similarly, Crawford et al. (2021), even increasing the

Table 4
In Silico functional information of *SERPINA6/1* locus variants available in RegulomeDB v2.1, CADD and Haploreg v4.2.

	RegulomeDB		*CADD*	HaploReg				
SNP ID	***Rank***	***Score***	**PHRED-*Score***	**Promoter Histone Marks Tissues**	**Enhancer Histone Marks Tissues**	**DNAse Tissues**	**Motifs Changed**	**eQTL Hits**
rs11621961	**1 f**	**0.55436**	**2.448**	**-**	-	**-**	**1 altered motif**	***IFI27L1* in lymphoblastoid cell line**
rs11629171	1 f	0.22271	0.557	-	2 tissues	1 tissue	-	*SERPINA1* in whole blood
rs7161521	1 f	0.66703	3.937	2 tissues	7 tissues	-	4 altered motifs	*SERPINA1* in lymphoblastoid cell line
rs941599	1 f	0.55436	1.037	2 tissues	4 tissues	-	2 altered motifs	*SERPINA1* in lymphoblastoid cell line *SERPINA6* in liver
rs2281518	1 f	0.55436	6.883	-	-	-	1 altered motif	*SERPINA1* in lymphoblastoid cell line
rs12589136	1 f	0.55436	0.963	-	1 tissue	-	3 altered motifs	*SERPINA1* in lymphoblastoid cell line *SERPINA1* in whole blood
rs1956174	1 f	0.22271	6.509	-	1 tissue	-	10 altered motifs	*PPP4R4* in EBV transformed lymphocytes *SERPINA1* in lymphoblastoid cell line *PPP4R4* in lymphoblastoid cell line
rs2749530	**1 f**	**0.22271**	**1.058**	**-**	**1 tissue**	**-**	**2 altered motifs**	***SERPINA2P* in whole blood** ***SERPINA1* in whole blood**
rs2749529	**7**	**0.51392**	**2.228**	**-**	**-**	**-**	**2 altered motifs**	***SERPINA2P* in whole blood** ***SERPINA1* in whole blood**
rs4905188	1 f	0.55436	5.206	-	8 tissues	1 tissue	1 altered motif	*SERPINA1* in lymphoblastoid cell line
rs2749527	1 f	0.55324	2.784	-	4 tisses	-	3 altered motifs	*SERPINA1* in whole blood
rs12588394	1b	0.92576	0.364	-	5 tissues	3 tissues	3 altered motifs	*SERPINA1* in lymphoblastoid cell line
rs3819333	1 f	0.55436	0.009	-	2 tissues	3 tissues	3 altered motifs	*SERPINA1* in lymphoblastoid cell line
rs941595	1 f	0.55324	2.220	-	1 tissue	-	-	*SERPINA1* in lymphoblastoid cell line
rs1950652	1 f	0.55436	0.913	-	4 tissues	1 tissue	4 altered motifs	*SERPINA1* in lymphoblastoid cell line
rs3762132	**1 f**	**0.55436**	**2.294**	**-**	**4 tissues**	**1 tissue**	**2 altered motifs**	***SERPINA2P* in whole blood** ***SERPINA1* in whole blood**
rs3762130	1 f	0.55436	0.725	-	3 tissues	-	1 altered motif	*SERPINA1* in lymphoblastoid cell line
rs3748319	1 f	0.55436	0.593	-	3 tissues	-	4 altered motifs	*SERPINA1* in lymphoblastoid cell line
rs1243171	**1 f**	**0.19549**	**0.601**	**-**	**1 tissue**	**-**	**-**	***SERPINA2P* in whole blood** ***SERPINA1* in whole blood**

Single nucleotide polymorphism (SNP), CADD: combined annotation dependent depletion CADD, Expression quantitative trait loci (eQTL), Interferon alpha inducible protein 27 like 1 (*IFI27L1*), Serpin family A member 1 (*SERPINA1*), Serpin family A member 2 gene/pseudogene (*SERPINA2P*), Protein Phosphatase 4 Regulatory Subunit 4 (*PPP4R4*).

sample size for 25,314 reinforced the association between genetic variations at the *SERPINA6/1* locus on chromosome 14 and morning plasma cortisol levels, without identifying new loci. In our sample, we did not identify significant additive effects from thousands of SNPs with small impact as the primary contributors to HCC. However, the limited size of our sample restricted statistical power in this aspect of the analysis, thereby preventing drawing definitive conclusions. The gene-based analysis in our study did not identify significant associations, aligning with the candidate SNP results, which suggest that a single SNP may play a pivotal role within the locus. While we could not attribute the observed effects to the entire locus, the identified SNP is located within the gene and is potentially functionally relevant. Other SNPs lost significance after correction for multiple testing, likely reflecting limited statistical power. These findings underscore the need for further research to thoroughly explore the relationship between the entire locus and HCC.

Our functional *in silico* analyses investigates the hypothesis that the tested variants might be relevant for *SERPINA6/1* locus regulation. Given that these variants are located in non-coding regions of the genome, their influence is likely exerted through the modulation of gene expression. The *in silico* investigations identified several markers indicative of gene regulation, including promoter and enhancer histone marks, DNase I hypersensitive sites, motif alterations, and eQTL hits across various tissues such as liver, breast, skin, and blood. Specifically, our functional analysis suggests that the rs11621961 variant may have a high probability of functionality and a regulatory role, as suggested by RegulomeDB scores. This SNP appears to modify the SMAD motif, which is integral to the signaling pathways of the transforming growth factor beta (TGF-β) superfamily. This association further underscores their potential role in gene expression regulation, suggesting that these SNPs may contribute to the genetic underpinnings of cortisol regulation and potentially other related physiological processes.

Current research indicates a complex relationship between HCC and self-reported stress, with most studies showing no significant correlation (Gidlow et al., 2016; Bryson et al., 2021). This suggests that the link between physiological stress markers and subjective experiences is nuanced, being influenced by individual perceptions, reporting differences, and the nature and duration of stressors. Our findings add a valuable perspective by suggesting a common biological foundation for various cortisol sample types, proposing HCC as a viable biomarker for

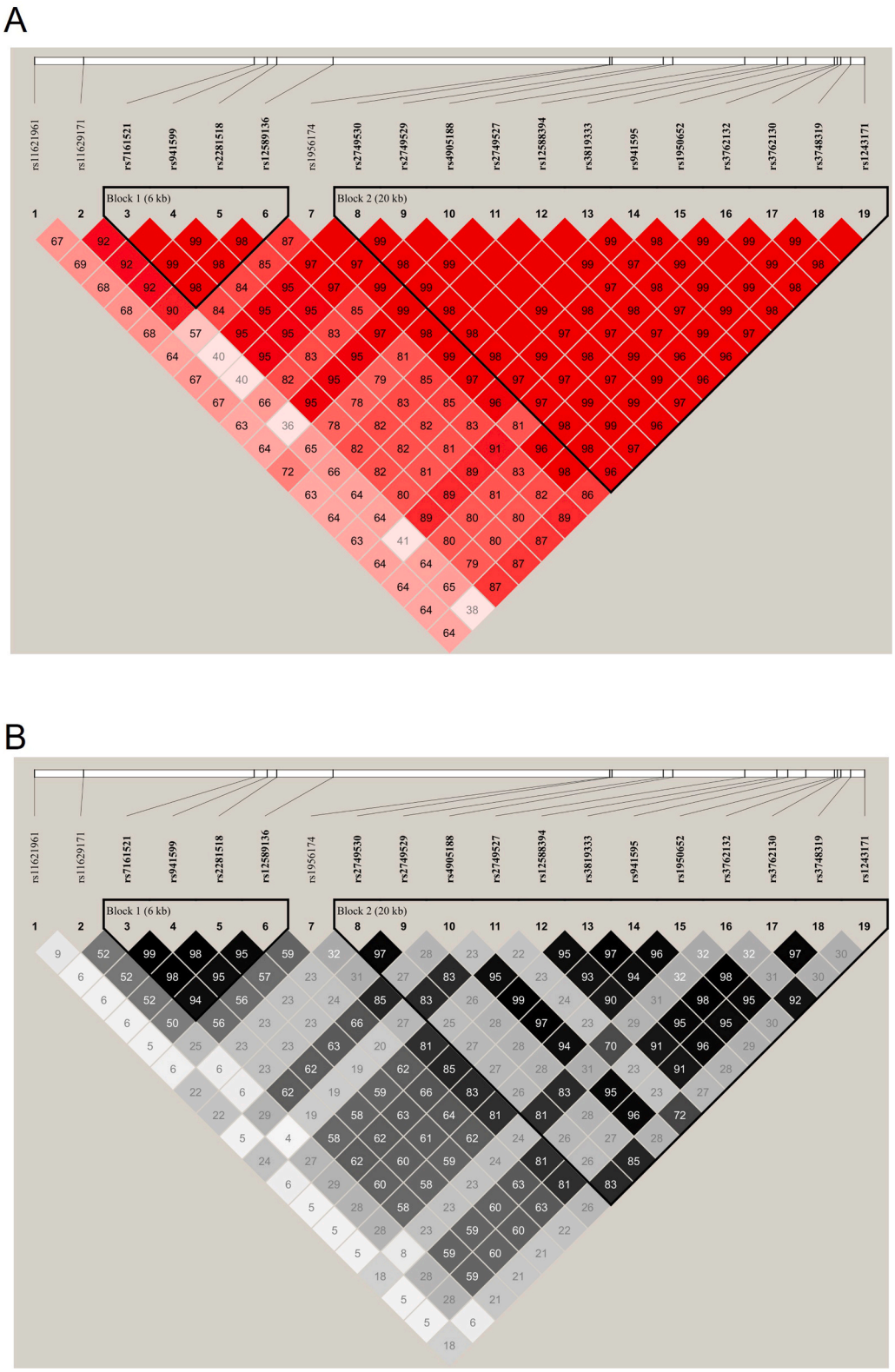

Fig. 1. Linkage disequilibrium (LD) plot with D´(A) and r^2 (B) parameters generated using Haploview 4.2. The plot identified two haplotype blocks that ranged in size from 6 kb to 20 Kb of the *SERPINA6/1* locus.

assessing HPA axis activity. HCC is advantageous as it reflects cumulative cortisol levels, providing a more comprehensive assessment of physiological stress responses than single-time-point measures or subjective reports. By capturing long-term cortisol exposure, HCC enhances stress research, offering valuable insights into the extended health impacts of stress and complementing self-reported data (Stalder and Kirschbaum, 2012; Vanaelst et al., 2012).

Given the adolescent sample in this study, it is important to acknowledge that recent research highlights puberty as a period of heightened HPA-axis plasticity. During this stage, the impact of early-

life stress on cortisol regulation may undergo significant changes. The rise in sex hormones, such as DHEA and testosterone, is thought to play a pivotal role in recalibrating cortisol regulation (King et al., 2020; Ruttle et al., 2015). King et al. (2020) observed that puberty initiates coordinated hormonal activity, establishing links between cortisol, DHEA, and testosterone. Similarly, Ruttle et al. (2015) reported that cortisol-DHEA coupling strengthens with age, while cortisol-testosterone coupling transitions from positive at age 11 to negative by ages 13 and 15. Moreover, alterations in cortisol levels during early life can have lasting repercussions on adult health, given cortisol's critical role in stress regulation and its associations with physical and mental health outcomes, as well as attenuated stress responses over time (Kornelsen et al., 2019). Future research addressing these dynamics is essential for advancing our understanding of HPA-axis changes during puberty and their implications for long-term health.

The literature indicates that factors beyond genetics, such as hair pigmentation and type, can influence hair cortisol concentration. Rippe et al. (2016) and Neumann et al. (2017) previously reported that hair color was associated with cortisol and cortisone levels, specifically that higher cortisol levels were found in darker hair (Rippe et al., 2016; Neumann et al., 2017). This suggests that hair pigmentation might impact cortisol extraction efficiency, with observed differences potentially reflecting localized effects. Furthermore, hair color and type are closely related to genetic ancestry, mirroring genetic variances in subpopulations (Neumann et al., 2017). In our admixed population, featuring diverse hair types and colors, these factors could potentially influence our results. However, when conducting sensitivity analyses, adjusting for these variables as potential confounders, our genetic association findings remained robust and significant, underscoring the genetic influence in our study. The only variation observed was in the association between candidate SNPs at the *SERPINA6/1* locus and HCC. When restricting analysis to corticosteroid use, rs11621961 lost significance after adjusting for multiple tests. We hypothesize that this outcome may be attributed to the reduced sample size when considering all covariates in the model.

Our results should be interpreted within the context of certain limitations. First, the follow-up rate at 15 years was relatively low compared to similar studies. This was primarily due to the interruption of our 15-year follow-up fieldwork in March 2020, a consequence of the COVID-19 pandemic. However, this interruption was not a major source of selection bias in the cohort at the 15-year follow-up. The sample included in our analysis appears generally representative of the entire cohort at baseline, but with differences in birth weight, prematurity rates and family income. Consequently, the results might not be generalizable to those specific groups. Not all participants had the required 3 cm of hair for cortisol analysis. The inclusion of individuals with shorter hair represents a limitation in assessing cumulative cortisol levels over the preceding three months. This methodological constraint is well recognized in studies utilizing hair cortisol measurements, particularly when shorter hair samples are analyzed. To account for this potential bias, hair length was included as a covariate in the sensitivity analysis, which yielded results consistent with our primary findings. Another important aspect to consider are the potential limitations arising from the lack of functional and genomic reference panels for admixed populations. This gap is significant because SNPs identified in GWAS are predominantly discovered in populations of European descent. Admixed populations can exhibit distinct allele frequencies, LD patterns, and differing effect alleles. Moreover, the cortisol discovery GWAS was conducted using European samples and in morning plasma cortisol, which served as the base for constructing the PGS in the Pelotas Cohort. Considering that in admixed populations there might be variations in effect sizes and LD structures, this could lead to an underestimation on the studied outcomes. This scenario highlights a potential area for future research to address these disparities. It is important to acknowledge that poverty and financial strain are well-established contributors to increased stress, which may, in turn, affect cortisol levels. Future research investigating their influence on cortisol variability is essential to advance our understanding of the underlying biological and social mechanisms. Finally, given potential variations in SNP effects and directions across populations, for some SNPs, the study was underpowered due to their frequencies and effect sizes. This limited statistical power may have reduced our ability to detect certain associations, thereby constraining the strength of our conclusions.

Among the strengths of our study is the innovative inclusion of hair cortisol data from adolescence, coupled with the identification of a genetic component in cortisol variation and the HPA-axis pathway through robust analyses. This approach of examining the genetics of HCC is quite new in the field, marking our research as a significant advance in understanding these mechanisms. Additionally, the design of the 2004 Pelotas Birth Cohort study, a population-based sample with high follow-up rates, enhances the credibility and relevance of our findings. Another important strength of our study is the investigation of an ethnically admixed population from a middle-income country. This aspect allows us to explore genetic associations in a different ancestry and socioeconomic context, diverging from most studies published on this topic. Moreover, detailed evaluations within population cohort samples, as conducted in our study, are infrequently reported in the literature, thereby adding substantial value and robustness to our findings. We also performed sensitivity analyses to avoid confusion in the effect measures.

5. Conclusion

In conclusion, our study demonstrated that genetic variation of rs11621961 within the *SERPINA6/1* locus is associated with differences in HCC among adolescents from the 2004 Pelotas Birth Cohort. Importantly, the investigation of our ethnically admixed population from a middle-income country allows us to explore genetic associations in a different ancestry and socioeconomic context, diverging from most studies published on this topic.

Further investigations are required to clarify the potential regulatory effects of this variant on CBG expression or its characteristics in cortisol binding. Such studies will help improve the significance of the *SERPINA6/1* locus in hair cortisol. Additionally, future research should focus on understanding the role of the *SERPINA6/1* locus in cortisol regulation and its connection to diseases, especially through gene-environment interactions studies. Longitudinal studies assessing how variations in this locus affect cortisol over time, particularly under chronic stress are essential for future learning.

Clinically, interventions targeting cortisol modulation in individuals with specific *SERPINA6/1* variants could offer strategies to reduce stress-related diseases, contributing to personalized treatments and a better grasp of *SERPINA6/1*'s role in disease.

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Ethical statement

The research protocol of the 2004 Pelotas Birth Cohort 15 years follow up was approved by the Research Ethics Committee of the Faculty of Medicine at the Federal University of Pelotas and by the Ethics Committee for the Analysis of Research Projects of the University of São Paulo under the number of approvals 40602124 and 889,753. The Brazilian National Commission for Research Ethics also approved the use of genomic data. Written informed consent was obtained from the mothers or legal guardians, and adolescents also signed an informed consent form.

CRediT authorship contribution statement

de Almeida Júlia Oliveira: Writing – review & editing, Investigation. **Gonzalez Andrea:** Writing – review & editing, Investigation. **Santos Iná S.:** Writing – review & editing, Project administration, Investigation. **Barros Fernando:** Writing – review & editing, Investigation. **Oliveira Isabel O.:** Writing – review & editing, Investigation. **Matijasevich Alicia:** Writing – review & editing, Project administration, Investigation. **Tovo-Rodrigues Luciana:** Writing – review & editing, Project administration, Investigation. **Camerini Laísa:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Murray Joseph:** Writing – review & editing, Investigation.

Declaration of Competing Interest

The authors declare no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2025.107470.

Data availability

Due to the internal policies of the 2004 Pelotas Birth Cohort, data will be made available upon request.

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