**Genetic Confounding in Bullying Research: Causal Claims Revisited**

**Supplemental materials**

**Supplemental material 1**

**Details Genotyping, Imputation, and Generation of Summary Statistics That are Independent of the TRAILS Sample**

**Genotyping and Imputation**

Blood samples of the TRAILS participants were collected at T3 for the population cohort and at T2 for the high-risk cohort. Participants who did not give blood were asked to provide buccal cells. DNA was extracted from blood samples (*n* = 1,565) or, in a small proportion of samples, buccal swaps (Cytobrushw; *n* = 360) using a manual salting out procedure (Miller et al., 1988). Genotyping was performed on the Golden Gate Illumina BeadStation 500 and the Infinium™ HumanCytoSNP-12 v2.1 BeadChip platforms (Illumina Inc., San Diego, CA), according to the manufacturer’s protocols. These datasets were merged and checked for genotype concordance. One SNP showed > 5% mismatches and was excluded from the Golden Gate dataset after checking the minor allele frequency with HapMap. All DNA samples were retained (sample concordances were all > 95%). Genetic variants that had > 5% missing data, had a minor allele frequency < 1%, or that deviate significantly from the Hardy-Weinberg equilibrium (*p* < 10-6) were excluded. In addition, DNA samples that had > 5% missing data, were too heterogeneous, were duplicated or related, or were from non-European descent (as determined by principal components analysis of our samples combined with all 1000G samples) were removed. Genotypes were next imputed using the Haplotype Reference Consortium’s global reference panel on the Michigan Imputation server (Das et al., 2016; McCarthy et al., 2016).

**Detailed Procedure for Generating Summary Statistics That are Independent of the TRAILS Sample**

Data from 1,226 TRAILS participants have been used in the genome-wide association study (GWAS) on Lifetime cannabis use that was one of the seven GWASs included in the meta-GWAS on Externalizing problems. To solve this dependence between meta-GWAS and validation sample, we used R package MetaSubtract version 1.60 (Nolte, 2020a) to subtract the results of the validation cohort from the meta‐GWAS results analytically and produce meta-GWAS summary statistics that are independent of the TRAILS sample (Nolte, 2020b). In this particular case of a meta-GWAS GSEM analysis combining seven different GWAS, adjusting the meta-GWAS externalizing summary statistics for the TRAILS Cannabis GWAS without taking into account the factor loading would be over-adjusting. Therefore, all estimates and standard errors from the TRAILS Lifetime cannabis GWAS were first multiplied by the factor loading of lifetime cannabis use on externalizing problems (0.77) from the meta-GWAS (Karlsson Linnér et al., 2021) and these effects were subsequently used in MetaSubtract to adjust for the inclusion of TRAILS in the meta-GWAS.

**Supplemental material 2**

**Difference Between Genetic Confounding and Genetic Overlap**

**Figure S1**

*The Association Net of Genetic Confounding is Only Adjusted for Direct Genetic Effects*



**Bullying victimization**

**Internalizing problems**

**Figure S2**

*The Association Net of Genetic Overlap is Adjusted for Mediated Genetic Effects*

**Internalizing problems**

**Bullying victimization**



**Supplemental material 3**

**Details *GsensY* Models**

The *GsensY* method (Pingault et al., 2021) works as follows: In a first linear structural equation model, it is tested whether the polygenic score of the outcome trait confounds the effect between exposure and outcome treating the genetic factor (polygenic score) as a mediator. The remaining effect after adjusting for genetic confounding is also estimated. A mediation model can be used because confounding and mediation are statistically equivalent in linear structural equation modelling (MacKinnon et al., 2000). In a second and third model, it is tested to what extent the association between exposure and outcome decreases if we control for polygenic scores that more fully capture the genetic effect. This method entails combining polygenic scores with other heritability estimates, i.e., SNP based heritability estimates and twin based heritability estimates. To this end, a latent variable G\* is added to the model to capture the heritability of the outcome under the hypothetical scenarios in which the polygenic score is inflated to the size of SNP heritability or twin heritability. Figures S3 and S4 display the details.

**Figure S3**

*Conceptual and Statistical models GsensY*

*β*XY

*β*GY

Y

X

*β*GX

G

*Note.* Adapted from Pingault et al. (2021). The solid lines represent the conceptual model of genetic confounding. In the statistical model, genetic confounding is estimated by treating the genetic factor (G) as a mediator (see dashed line): the confounding effect is the indirect effect of exposure X on outcome Y through genetics (G): *β*XG*β*YG.

**Figure S4**

*Observed and Hypothetical Models in GsensY*

*β*XY

1

G

G\*

Y

X

*β*G\*X

*β*G\*Y

*β*G\*G

*Note.* Adapted from Pingault et al. (2021). The latent variable G\* is used to capture the heritability of the outcome (Y) under the hypothetical scenarios. Estimates of SNP heritability and twin heritability are single values retrieved from other studies and they were added to the model as part of a constraint: *β*G\*Y + *β*G\*X*β*XY = √(*h2*y). In the SNP heritability model *h2*yequals the SNP heritability estimate and in the twin heritability model *h2*yequals the SNP heritability estimate. In the observed model in which only polygenic scores are used and no other heritability estimates, the path *β*G\*G is always 1, because *h2*y is set to be equal to the explained variance in outcome Y of the genetic factor G (the polygenic score of the outcome trait). In the hypothetical models, *h2*yis the SNP heritability (SNP model) or the twin heritability (twin model) of outcome Y. This means that *β*G\*G is no longer fixed to 1 and is now estimated. *GsensY* requires as input: (1) bivariate correlations between X, Y, and G from the researcher’s own sample; (2) a SNP heritability estimate and a twin heritability estimate from another study to estimate G\* in the SNP heritability and twin heritability models.

**Supplemental material 4**

**Table S1**

*Details Distribution Bullying Involvement*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Bullying involvement per wave |  | Bullying involvement waves T1 and T2 combined |  |
|  |  | Not victimized  | Somewhat or sometimes victimized  | Severely or frequently victimized | Missings | Not victimized at either wave | Somewhat or severely victimized at one wave  | Somewhat or severely victimized at both waves | Missings |
| Self-report Victimization | T1 | 1029 (65%) | 449 (28%) | 108 (7%) | 18 | 908 (58%) | 435 (28%) | 220 (14%) | 41 |
| T2 | 1253 (79%) | 279 (18%) | 49 (3%)  | 23 |  |  |  |  |
|  |  | Did not bully others | Bullied others somewhat or sometimes | Bullied others severely or frequently | Missings | Did not bully at either wave | Bullied others somewhat or severely at one wave  | Bullied others somewhat or severely at both waves | Missings |
| Self-report Bullying | T1 | 1105 (70%) | 455 (29%) | 20 (1%) | 24 | 987 (63%) | 462 (30%) | 109 (7%) | 47 |
| T2 | 1365 (86%) | 206 (13%) | 10 (1%) | 23 |  |  |  |  |