Journal of Psychiatric Research 160 (2023) 217–224

Contents lists available at ScienceDirect

Journal of Psychiatric Research

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

Epigenetic changes in the CYP2D6 gene are related to severity of suicide attempt: A cross-sectional study of suicide attempters

Adrian E. Desai Boström [a,b,c,*], Esmail Jamshidi [a,d], Diana-Maria Manu [e], Lara Kular [f], Helgi B. Schiöth [e], Marie Åsberg [g], Jussi Jokinen [a,g]

[a] Department of Clinical Sciences/Psychiatry, Umeå University, Umeå, Sweden
[b] Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet, & Stockholm Health Care Services, Region Stockholm, Karolinska University Hospital, SE-171 76, Stockholm, Sweden
[c] Department of Women's and Children's Health/Neuropediatrics, Karolinska Institutet, Stockholm, Sweden
[d] Stockholm Health Care Services, Region Stockholm, Stockholm, Sweden
[e] Department of Surgical Sciences, Functional Pharmacology and Neuroscience, Uppsala University, Uppsala, Sweden
[f] Department of Clinical Neruoscience/Neuro, Karolinska Institutet, Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden
[g] Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden

ARTICLE INFO

Keywords:
Suicide
Suicide prevention
DNA methylation
Psychiatry
CYP2D6
cg07016288

ABSTRACT

Background: The ability to accurately estimate risk of suicide deaths on an individual level remains elusive.
Methods: This study reports on a case-control study set-up from a well-characterized cohort of 88 predominantly female suicide attempters (SA), stratified into low- (n = 57) and high-risk groups (n = 31) based on reports of later death by suicide, as well as degree of intent-to-die and lethality of SA method. We perform an unbiased analysis of 12,930 whole-blood derived CpG-sites (Illumina Infinium EPIC BeadChip) previously demonstrated to be more conciliable with brain-derived variations. The candidate site was validated by pyrosequencing. External replication was performed in (1) relation to age at index suicide attempt in 97 women with emotionally unstable personality disorder (whole-blood) and (2) death by suicide in a mixed group of 183 prefrontal-cortex (PFC) derived samples who died by suicide or from non-psychiatric etiologies.
Results: CYP2D6-coupled CpG-site cg07016288 was hypomethylated in severe suicidal behavior ($p < 10E-06$). Results were validated by pyrosequencing ($p < 0.01$). Replication analyses demonstrate hypomethylation of cg07016288 in relation to age at index SA in females ($p < 0.05$) and hypermethylation in PFC of male suicide completers ($p < 0.05$).
Limitations: Genotyping of *CYP2D6* was not performed and CpG-site associations to gene expression were not explored.
Conclusions: CYP2D6-coupled epigenetic markers are hypomethylated in females in dependency of features known to confer increased risk of suicide deaths and hypermethylated in PFC of male suicide completers. Further elucidating the role of *CYP2D6* in severe suicidality or suicide deaths hold promise to deduce clinically meaningful results.

1. Introduction

Suicide is a major cause of mortality and years-lost worldwide and a major public health concern. Globally, it is estimated that 700,000 deaths each year are caused by suicide (WHO)(World Health Organization (WHO), 2021). Endeavors have been made for over a century to determine major risk factors and causes of suicide. These endeavors were initially primarily based on social research and have since been supplemented with biological and neuropsychiatric oriented models. Complex epigenetic models and genome-wide association studies (GWAS) have been a particular focus of preventive suicidology over the past decade. Despite scientific progress, the ability to accurately estimate suicide risk on an individual level remains elusive and has not significantly improved in the last half century(Mishara and Weisstub, 2021). Theories have been put forth that suicide prediction models are complicated by the complex and varied underlying pathophysiology,

* Corresponding author. Umeå University, 901 87, Umeå, Sweden.
E-mail address: adrian.bostrom@umu.se (A.E.D. Boström).

https://doi.org/10.1016/j.jpsychires.2023.02.025
Received 21 October 2022; Received in revised form 7 February 2023; Accepted 22 February 2023
Available online 24 February 2023

encompassing both hereditary, environmental and emotional inputs (Mishara and Weisstub, 2021). However, recent progress in suicide research implicate that suicide attempters exhibiting high intent to die or choosing more lethal methods are more neurobiologically comparable to suicide victims(Jokinen et al., 2010a,b ; Stefansson et al., 2012; Stenbacka and Jokinen, 2015)'(Jollant et al., 2005). These presumptions indicate additional knowledge can be gained from deep phenotyping of suicidal subjects. In a wider sense, they promote the intriguing prospect of uncovering biomarkers for severe suicidal behavior by identifying distinct neurobiological underpinnings of this subcategory. Arguably, such endeavors hold promise to uncover objective, reliable and complementary biomarkers to aid in efforts to distinguish suicide attempters evincing a high risk of later death by suicide.

A previous epigenome-wide DNA-methylation meta-analysis identified distinct prefrontal cortex (PFC)-derived DNA methylation within the *PSORS1C3* gene by comparing suicide cases to non-suicidal sudden-death controls(Policicchio et al., 2020a). Other epigenome-wide association studies (EWAS) investigating confirmed suicide cases – with DNA methylation measured from white blood cells(Bani-Fatemi et al., 2020, 2021) and hippocampus(Kouter et al., 2019) - report varying results and are burdened by limited sample sizes. While smaller sample studies allow for the identification of DNA methylation variations with large effect size, recent research informs us that such studies should be inadequately powered to comprehensively exclude the involvement of additional DNA methylation regions(Mansell et al., 2019). Jokinen et al. investigated hypothalamic-pituitary-adrenal (HPA)-axis coupled methylation sites in a cohort of suicide attempters measured in whole-blood, stratified into low- and high-risk groups based on the severity of suicidal behavior. This study detected a corticotropin releasing hormone (CRH)-coupled DNA methylation site to be hypomethylated in the high-risk group of suicide attempters(Jokinen et al., 2017a,b).

The main goal of the study was to identify blood-based epigenetic biomarkers for severe suicidality (with higher risk of later suicide). In a well-characterized cohort of 88 suicide attempters, 31 subjects that used a violent method of attempted (i.e., hanging, shooting, or drowning) (Träskman et al., 1981), exhibiting a high intent-to-die as measured by the Freeman scale(Freeman et al., 1974), or having later death by suicide were classified as high-risk. Whole blood derived DNA methylation markers - whereby individual factors were previously shown to influence methylation levels to a greater extent than the tissue from which it was extracted – were investigated in relation to severity of suicide attempt. Candidate CpG-site/s were measured in the same cohort by pyrosequencing and assessed for replicability. An external study group consisting of 97 female emotionally unstable personality disorder (EUPD, previously borderline personality disorder) patients with severe suicidal behavior (a history of at least two recent SAs) was used to further investigate associations between candidate methylation sites and occurrence of later suicide (N = 8). Importantly, age at onset of first SA (index attempt [IA]) has previously been implicated as a marker to delineate SA subgroups(Menon et al., 2018), whereby adolescent IA has been associated with a substantially greater contribution of firearms and overall lethality(McKean et al., 2018). Thus, candidate methylation sites were contrasted between individuals with a history of suicide attempt in adolescence and adulthood, respectively. A secondary objective was to further explore any underlying neurobiological processes in brain that such biomarkers may elicit information of. Thus and subsequently, openly available data across three independent data sets was retrieved to further investigate candidate probes in postmortem extracted brain biopsies (prefrontal cortex – previously associated with epigenetic dysregulation in suicide completers(Murphy et al., 2017a; Nagy et al., 2017; Schneider et al., 2015)) from 88 suicide-completers compared to 95 non-psychiatric controls. As a final step - considering well-studied sex differences in suicide-related outcomes present in both adolescent and adult populations (putatively with distinct underlying neurobiological mechanisms(Ho et al., 2021)) - in each data set, the above analyses were re-performed exclusively in male (where available) and female subjects, respectively.

2. Methods

2.1. Characterization of the discovery group

2.1.1. Ethics and patient consent

Details on the cohort of death by suicides and stratification of subjects have been previously published(J. Jokinen et al., 2017). In brief, the study protocols were approved by the Regional Ethical Board in Stockholm, Sweden (Dnrs: 00–194,2015/1454.32) and the participants gave their informed consent to the study, which was conducted in accordance with guidelines and regulations.

2.1.2. Discovery cohort of suicide attempters

The study includes adult patients that were clinically assessed in 2000–2005 at a Suicide Prevention Clinic (Karolinska university hospital) after conducting acts of self-destruction with variable intention to die. Subjects documented with psychotic disorders, dementia, mental disability or (intravenous) substance abuse were excluded. To improve the clinical generalizability of the study, patients with a history of non-suicidal self-injury and/or non-intravenous substance abuse were not included in the participant exclusion criteria. After exclusion of subjects declining to participate (n = 50), not meeting inclusion criteria (n = 61) or an inability to attend clinical follow-up visits (n = 47), 100 patients were included in the study (33 men and 67 women). 26 subjects were medication-free at the time of assessment. The only medication reported in more than five individuals were antidepressants (Sertraline [n = 20], Citalopram [n = 12], Mirtazapine [n = 12], Venlafaxine [n = 9] and Fluoxetine [n = 7]). No individual subjects received lithium treatment (Sinai et al., 2009). DNA samples were extracted from 88 of these subjects. The information about suicide and cause thereof was available from matching unique identification numbers with the national Cause of Death register. Later death by suicide was established in four cases in the years 2004, 2006, 2007 and 2014, respectively - three of which from hanging and one from substance intoxication. Subjects that used a violent method of suicide attempt (i.e., hanging, shooting, or drowning) (Träskman et al., 1981), exhibiting a freeman scale of >6(Freeman et al., 1974), or having later died by suicide were classified as high-risk.

2.1.2.1. Blood sample collection. Peripheral blood specimens were collected according to standardized procedures. Study samples were required to be fasting at the time of blood sampling, which occurred in morning.

2.1.2.2. Methylation profiling and data processing. DNA was retrieved in using the phenol-chloroform method, after which bisulfite conversion was performed in accordance with the EZ DNA Methylation GoldTM kit (ZymoResearch, USA). DNA specimens were thereafter hybridized to the Illumina Infinium Methylation EPIC beadchip and the array was imaged in using the Illumina Iscan system (Illumina, San Diego, CA, USA), resulting in the quantification of methylation values at approximately 850,000 unique methylation identifiers across all samples. Preprocessing of methylation data included background correction, adjustment for methylation site measurement techniques (i.e., type I and type II probes), adjusting for any potential batch effects and the removal of putatively unreliable probes. DNA methylation data was corrected for in-silico generated surrogate measures of white blood cell type heterogeneity. Evaluation of sample outliers based on methylation data was performed by principal component analysis (PCA). Methylation preprocessing steps were performed in using R software, version 3.3.0, and the following bioconducor packages - minfi(Aryee et al., 2014), watermelon(Schalkwyk et al., 2013), sva(Leek et al., 2012), champ(Morris et al., 2014) and FactomineR(Lê et al., 2008). A detailed description of

the preprocessing steps has been previously published(J. Jokinen et al., 2017).

2.1.3. Annotation and selection of DNA methylation probes

The validity of implementing blood-based psychiatric EWAS studies to discern interindividual DNA methylation variation in inaccessible tissues where the main biological effects is presumed to occur has come under increasing scrutiny(Hannon et al., 2015). To improve transferability of any findings measured in whole blood to changes occurring in brain, we only considered the 22,458 CpG sites whereby Hannon et al. previously demonstrated that individual characteristics predicts more of the variance in DNA methylation than the tissue from which the DNA methylation was extracted(Hannon et al., 2015). We further limited the analysis to CpG sites located within 2000 bp of the transcriptional start site of the nearest gene according to Price et al. (2013), as these have been shown to especially influence transcription of the adjacent gene (Wagner et al., 2014). Following preprocessing procedures, a total of 12,930 such CpG-sites were included in the subsequent analysis.

2.1.4. Pyrosequencing

Validation of cg07016288 was performed using bisulfite (BS) pyrosequencing. Primers were designed using the PyroMark Assay Design software version 2.0 (Qiagen, Dusseldorf, Germany) (Fwd: AGTGTAGGTGGTTTTTTGGTT, 5′-biotinylated Rev: CCTAACCCTCCCTCTACAATT, sequencing: TTTTTATAGTTGTTTGTTTTGGGAA). Genomic DNA (500 ng) was bisulfite-converted using DNA methylation-Gold Bisulfite Kit (Zymo Research, Irving, CA, USA) and eluted in 25 μl of elution buffer. The converted DNA (~15 ng) was applied as a template in the PCR performed with the PyroMark PCR kit (Qiagen, Dusseldorf, Germany) following manufacturer's recommendations. To verify the efficiency and sensitivity of the pyrosequencing, unmethylated and fully methylated human BS-DNA samples (EpiTect PCR Control DNA Set, Qiagen, Dusseldorf, Germany) were included as controls. The entire PCR product, 4 pmol of the sequencing primer, and streptavidin sepharose high-performance beads (GR Healthcare) were used for pyrosequencing on the PSQ 96 system and PyroMark Gold 96 reagent kit (Qiagen). The PyroMark CpG software 1.0.11 (Qiagen, Dusseldorf, Germany) served for preliminary data analysis.

2.2. Characterization of replication data sets

2.2.1. Cohort of severely suicidal females with emotionally unstable personality disorder (EUPD group)

Details on the EUPD cohort have been previously published(Sinai et al., n.d., 2018; Zaboli et al., 2006) and are extensively described in Supplementary Material (including blood sample collection, methylation profiling and data processing).

2.3. Aggregated analysis of three data sets profiled post-mortem from the prefrontal cortex of suicide completers and non-psychiatric controls (Brain Suicide group)

Policicchio et al. recently performed a genome-wide DNA methylation meta-analysis of previously published studies in the brains of suicide completers and non-psychiatric controls(Policicchio et al., 2020b) and are described in more detail in Supplementary Material (including Methylation profiling and data processing steps, **Sections 1.1.1-2**).

2.4. Statistical analysis

2.4.1. Data analysis (discovery group)

The classification of violent and non-violent suicide attempts into low- or high-risk groups has been previously described in detail(J. Jokinen et al., 2017). In brief, dichotomization was performed with relevance concerning putative biological differences and subjects fulfilling any of the following criteria were classified as high-risk: violent suicide attempt method, or a high score in the Freeman Scale, or later death by suicide. Discriminating validity of the scale was considered very good in a previous study consisting of a large sample of attempted suicide subjects and suicide victims(Freeman et al., 1974). In accordance with the literature, non-violent attempts included substance intoxication or self-cutting of wrists, whereas violent attempts pertained to all other available suicide methods, i.e., attempted drowning, shooting, gassing or hanging(Träskman et al., 1981). Details pertaining to the classification of variables, data analysis and replication analyzes are available in Supplementary Material (**Sections 1.3.1-2**).

2.4.2. DNA methylation association study (discovery group)

Optimal co-variates were calculated to minimize risks of overfitting the subsequent model. Clinical variables previously shown to exhibit between-group differences with a p-value <0.05 were included as co-variates, i.e., gender and occurrence of death by suicide. For the subsequent analysis, methylation beta-values were log2-transformed to the more robust M-values(Du et al., 2010). The association analysis between DNA methylation and severity of suicide attempt were modelled by specific linear models using an empirical Bayes framework based on a moderated t-statistic – a method previously deemed adequate for epigenome-wide association studies(Rask-Andersen et al., 2016; Smyth, 2004). The analysis thus contrasted methylation M-values for each investigated CpG-site to the phenotype of interest (suicidal severity phenotype), adjusting for optimal co-variates (gender and occurrence of later suicide). To minimize risks of type I errors, p-values were adjusted for multiple-testing with the more stringent Bonferroni-method(Armstrong, 2014). Bonferroni-adjusted p-values were calculated by multiplication of the nominal p-value by the number of investigated CpG-sites (i.e., 12,930). In addition, epigenome-wide association studies (EWAS) are demonstrably sensitive to confounding from bias of test statistics and inflation. This could introduce spurious findings and are not completely abated by standard DNA-methylation preprocessing methods and/or confounder adjustment methods. The R/Bioconductor package BACON implements a Bayesian method based on the empirical null distribution – and is demonstrated from both simulated and real data to adequately control for bias and inflation in EWAS studies(van Iterson et al., 2017). To exclude potential unmeasured sources of confound, we therefore implemented the R/Bioconductor package BACON(van Iterson et al., 2017) to adjust the global regression results for estimated bias and inflation. P-values <0.05 after Bonferroni-, bias- and inflation adjustments were considered significant. Methylation beta-values of such significant methylation sites were illustrated in boxplot/s. To examine the impact of excluding the co-variate pertaining to individuals who later died by suicide, the prior analysis was reperformed, adjusting for gender as a co-variate. To assuredly exclude any bias from unadjusted for factors, post-hoc binomial logistic regression models were performed contrasting the phenotype of interest (suicidal severity phenotype) to cg07016288 methylation M-values and adjusting for age, age, alcohol or substance abuse and use of antidepressants. Methods pertaining to replication analysis (pyrosequencing) and replication cohort analyses (EUPD and Brain Suicide groups) are available in **Suppementary Materials** (**Sections 1.3.2-3**).

3. Results

3.1. Cohort description (discovery group)

31 patients (35%) were classified into the high-risk/severe attempt category and 57 (65%) into the low-risk group. The severe suicidal risk-group exhibited an overrepresentation of male participants ($p < 0.01$). By a significance level <0.05, other clinical outcome variables were largely comparable between the two groups ($p > 0.05$), i.e., BMI, occurrence of depression, SSRI medication usage, personality disorder, alcohol dependence or substance abuse. Patients from both groups

scored equally on the Karolinska Interpersonal Violence Subscales which measures both expressed and exposure to violent behavior during child- and adulthood(Jokinen et al., 2010) (Table 1).

Age of all participants ranged from 18 to 67, with a mean of 34 years (SD: 12.4). In the whole sample, occurrence of one or more axis I psychiatric diagnoses were evinced in 86% of study participants, including mood disorders (76%), anxiety disorder (6%), adjustment disorder (5%), and alcohol abuse disorder (4%). Comorbid substance-abuse disorder (in the majority alcohol dependency) was detected in 12% of samples, whereas 4% exhibited comorbid eating disorders. Approximately 28 per cent of participants presented with personality disorders.

3.2. Investigation of 12,930 less tissue-reliant methylation probes reveals hypomethylation of blood-based measurements of CYP2D6-coupled CpG-site cg07016288 in severe suicidal behavior

On the association analysis between whole-blood DNA methylation and suicidal behavior, we studied 12,930 CpG-sites deemed to be more swayed by intraindividual factors compared to tissue. In this analysis, methylation M-values were contrasted to suicidal phenotype, adjusting for potential confound from co-variates with significant ($p < 0.05$) between-group differences (gender and occurrence of later suicide). After adjustments for multiple-testing by the more stringent Bonferroni-method, *CYP2D6*-coupled CpG-site cg07016288 exhibited significant hypomethylation in dependency of suicidal severity. The genomic inflation factor lambda measured 1.035, indicating minimal inflation of test-statistics. After additional adjustment for potential unmeasured sources of bias and inflation, cg07016288 remained significantly hypomethylated after Bonferroni-correction of p-values (Bonferroni correction factor: 12,930)(Table 2.)(Fig. 1.). A post-hoc analysis was performed to examine the impact of excluding the covariate 'occurrence of later suicide' from our study. Our results indicate that the top finding remains consistent after removal of this covariate, at a Bonferroni-corrected p-value of 0.034 (Bonferroni correction factor: 12,930). In a subsequent post-hoc analysis, cg07016288 was confirmed as significantly hypomethylated in the severe suicidal phenotype as measured by binomial logistic regression models contrasting the phenotype of interest (suicidal severity phenotype) to cg07016288 methylation M-values and adjusting for age, alcohol or substance abuse and use of SSRI medication ($p = 0.0145$).

Table 1
Characteristics of subjects.

	Attempted suicide (n = 88)		
	High-risk group	Low-risk group	Statistics (*t*-test, Mann-Whitney *U* test, chisq-test), p-value)
N	31	57	
Age (years)	35.16 (12.3)	33.6 (12.2)	*ns*
Men: Women, n(%)	16(51.6): 15(48.4)	12 (21.1): 45 (78.9)	**6.92E-03**
BMI, mean (SD)	24.3 (4.6)	24.9 (4.3)	*ns*
Depression, n(%)	23 (74.2)	37 (64.9)	*ns*
Borderline personality disorder, n(%)	7 (22.6)	5 (8.8)	*ns*
Other personality disorder, n(%)	11 (35.5)	10 (17.5)	6.60E-02
Alcohol dependence, (n(%))	9 (29.0)	8 (14.0)	9.35E-02
Substance dependence, n(%)	6 (19.4)	9 (15.8)	*ns*
Completed suicide, n (%)	4 (12.9)	0 (0.0)	**1.25E-02**
SSRI medication, n(%)	19 (61.2)	26 (45.6)	*ns*
KIVS subscale, n(%)**			
[1]Expressed violent behavior during			
Childhood	0 (0.0)	1 (1.8)	*ns*
Adulthood	6 (19.4)	4 (7.0)	*ns*
[2]Exposure to violent behavior during			
Childhood	10 (32.3)	15 (26.3)	*ns*
Adulthood	15 (48.4)	19 (33.3)	*ns*

Values are shown as mean (SD) unless otherwise specified. P-values were calculated by means of *t*-test, Kruskal-Wallis' test or chi-squared test, contrasting values for subjects in the high-risk vs low-risk suicide attempt group. A one-tailed p-value <0.05 was considered significant.Abbreviations: KIVS, Karolinska Interpersonal Violence Scale; ns, not significant.

3.3. Hypomethylation status of cg07016288 in severe suicide attempters measured in whole blood was validated by pyrosequencing

For validation purposes, DNA-methylation levels of cg07016288 were measured by pyrosequencing in the Discovery cohort. 6 outlier samples were identified and excluded from subsequent analyses due to concerns over technical bias from measurement errors specific to pyrosequencing (described in section 2.6.3)(Supplementary Fig. 1), resulting in 53 and 27 individuals in the low- and high-risk group included in the subsequent analysis, respectively. Pearson correlations of cg07016288 Illumina EPIC and pyrosequencing measurements suggested moderate correlations ($r = 0.459$, $p < 0.0001$). The hypomethylation of cg07016288 in severe suicidal behavior was confirmed by pyrosequencing, as measured by independent-samples *t*-test, using one-tailed hypothesis testing assessing putative hypomethylation in the high-risk group ($p < 0.01$)(Supplementary Fig. 2.). The validity of the initial finding was further supported by multivariate binomial logistic regression models, contrasting suicidal risk group (low- or high-risk) to cg07016288 pyrosequencing measured methylation levels and the dichotomous co-variates gender (F/M) and occurrence of death by suicide (Y/N)($p < 0.01$)(Supplementary Table 1.).

3.4. Methylation of CYP2D6-coupled probe cg07016288, which is hypomethylated in severe suicidal behavior, is lower methylated in female EUPD patients with a history of first suicide attempt in youth (EUPD group)

The 97 EUPD participants were all females with a mean age and BMI of 29.4 years (SD = 7.6) and 24.5 kg/m^2 (SD = 4.7), respectively. Detailed characteristics of the EUPD group are available in **Supplementary Material** (**Section 2.1**) and Supplementary Table 3. Cg07016288 methylation levels were independent of occurrence of later suicide ($n = 8$, $p > 0.1$). However, history of a suicide attempt in adolescence was associated with Cg07016288 hypomethylation, when compared to subjects with a first suicide-attempt in adulthood ($p = 0.02966$) – as measured by one-sided *t*-tests in EUPD-subjects ($n = 97$) (Supplementary Fig. 3.). These findings were borderline-significant in subsequently performed multivariate binomial logistic regression models adjusted for optimal co-variates (occurrence of active MDD episode) ($p = 0.0815$)(Supplementary Table 4.). Cg07016288 methylation levels were unrelated to age, BMI and the use of SSRI medication ($p > 0.05$)(data not shown) – as measured by Pearson correlations (continuous variables, i.e., age and BMI) and chi squared-tests (dichotomous variables, i.e., medication usage frequencies).

3.5. Cg07016288 is borderline hypermethylated in prefrontal cortex of suicide completers compared to controls (Brain Suicide group)

Details of the three studies have been previously published(Guintivano et al., 2013; Kozlenkov et al., 2017; Murphy et al., 2017b; Viana et al., 2017) and presented at an aggregate level(Policicchio et al., n.d.). A detailed description of the characteristics of the Brain Suicide group is available in Supplementary Materials (**Section 2.2**).

In the aggregated data set, cg07016288 methylation M-values were borderline significantly hypermethylated in dependency of suicide ($\beta = 1.957$, $p = 0.0503$) – as measured by binomial logistic regression models adjusted for age, gender, neuronal components, sample (BA11, BA25 or

Table 2
Suicide attempt severity associated methylation changes in probes where individual predicts more of the variance than tissue.

Gene	Transcript	Illumina ID	% DNA Methylation (SD)	High-risk	logFC	p	p (Bonf.)	p_{BC}	p_{BC} (Bonf.)
			Low-risk						
CYP2D6	AY663390	cg07016288	81.1 (4)	78.7 (4.3)	−0.39	2.66E-06	3.44E-02	3.61E-06	4.67E-02
PPIEL	NR_003929	cg10243676	52.5 (4)	56.3 (4.9)	0.21	1.35E-05	*ns*	1.52E-05	*ns*
FAM212B	NM_198,926	cg23955903	2.3 (0.4)	2.6 (0.5)	0.17	5.18E-05	*ns*	5.71E-05	*ns*
C13orf39	NM_001,010,977	cg19359983	88.7 (4.9)	85.9 (5.6)	−0.57	7.26E-05	*ns*	9.09E-05	*ns*
RAE1	NM_003610	cg07931024	89 (3.4)	86.4 (3.4)	−0.45	9.49E-05	*ns*	1.18E-04	*ns*
ZNF133	AK316386	cg16872106	16 (3.7)	19.1 (5.7)	0.40	9.48E-05	*ns*	1.03E-04	*ns*
GPR108	NM_001,080,452	cg17223624	4.9 (1)	4.4 (0.7)	−0.20	1.24E-04	*ns*	1.53E-04	*ns*
LOC285441	NR_033,901	cg21004358	81.5 (8.9)	75.4 (7.2)	−0.71	1.26E-04	*ns*	1.55E-04	*ns*
CCDC42	NM_001,158,261	cg03115937	4.4 (0.6)	4.1 (0.8)	−0.17	1.34E-04	*ns*	1.66E-04	*ns*
APRT	NM_000485	cg07769176	2.8 (0.8)	3.3 (0.8)	0.31	1.87E-04	*ns*	2.01E-04	*ns*

Robust linear regression models of methylation M-values to a binary outcome variable of severity of suicide attempt (high/low-risk), adjusting for gender and occurrence of completed suicide. 12,930 individual CpG-sites whereby the variance of methylation levels better predicted by individual factors compared to tissues sampled. P-values were corrected for multiple testing using the Bonferroni-method. Correction for inflation and estimated bias was made using the package 'Bacon' for R statistics.Abbreviations: % DNA Methylation (SD), mean beta value percent DNA methylation (standard deviation); logFC, log fold change of M-values; p, p-value; pbc, p-values after bacon adjustment for inflation and bias; p_{bc} (Bonf.), Bonferroni-adjusted p-values after bacon adjustment for inflation and bias.

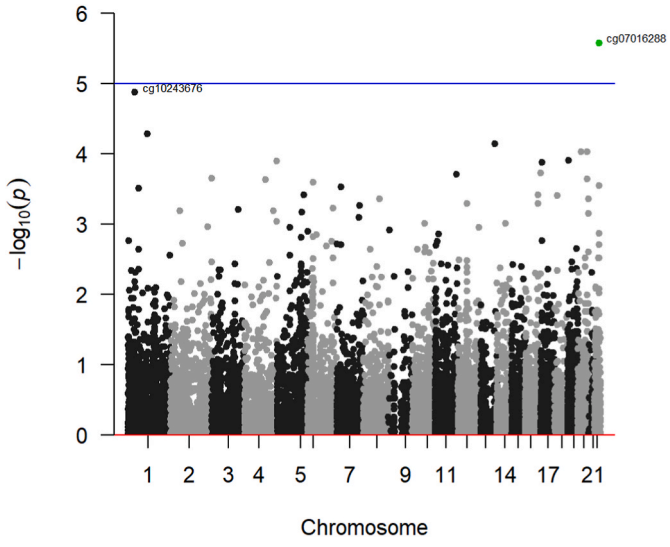

Fig. 1. Manhattan plot of the association analysis between whole-blood DNA methylation and suicidal behavior, studying 12,930 CpG-sites deemed to be more swayed by intraindividual factors compared to tissue. In this analysis, methylation M-values were contrasted to suicidal phenotype, adjusting for potential confound from co-variates with significant ($p < 0.05$) between-group differences (gender and occurrence of later suicide). After adjustments for multiple-testing by the more stringent Bonferroni-method, *CYP2D6*-coupled CpG-site cg07016288 exhibited significant hypomethylation in dependency of suicidal severity. The genomic inflation factor lambda measured 1.035, indicating minimal inflation of test-statistics. After additional adjustment for potential unmeasured sources of bias and inflation, cg07016288 remained significantly hypomethylated after Bonferroni-correction of p-values (Bonferroni correction factor: 12,930).

simply neuronal when not detailed) and a multi-factorial variable representing the varying datasets from which the data was extracted. Subsequently, the group was stratified by gender and analyzed separately (Table 3.). By the same analysis (excluding model gender adjustment) in 80 female and 103 male subjects, respectively, cg07016288 methylation M-values were not associated to cause of death ($p > 0.05$). Post-hoc independent samples *t*-tests indicated that male subjects were the driving factor behind the association of hypermethylation ($p = 0.0489$) – an association which was not observed in female samples ($p > 0.1$) (Fig. 2.).

Table 3
Binomial logistic regressions contrasting cause of death (suicide or non-psychiatric control) to cg07016288 methylation levels (n = 183).

	Estimate	Std. Error	*t*-value	*p*
Intercept	−5.0	2.3	−2.1	0.0343
Cg07016288	6.5	3.3	2.0	0.0503
Age	0.0	0.0	0.4	*ns*
Gender	0.8	0.3	2.3	0.0219
Experimental control variable 1	−0.4	1.0	−0.4	*ns*
Experimental control variable 2	−0.1	0.6	−0.2	*ns*
Neuronal components	0.5	1.0	0.5	*ns*
Tissue control variable (BA25/BA11/ "Neuronal")	−0.7	0.4	−1.6	*ns*

Results presented from the aggregated data set (n = 183). Binomial logistic regression models contrasting cause of death (suicide or non-psychiatric control) to cg07016288 methylation M-values and adjusted for age, gender, neuronal components, sample (BA11, BA25 or simply neuronal when not detailed) and a multi-factorial variable representing the varying datasets from which the data was extracted. Subsequently, the group was stratified by gender and analyzed separately.Abbreviations: p, p-value.

4. Discussion

Investigation of 12,930 whole-blood derived DNA methylation probes more conciliable with brain-derived variations revealed *CYP2D6*-coupled CpG-site cg07016288 to be significantly hypomethylated in dependency of severe suicidal behavior after stringent adjustments for multiple-testing, bias, and inflation. The hypomethylated status of the identified probe was validated by pyrosequencing-derived measures in both univariate and multivariate analyses. In an independent cohort of severely suicidal female EUPD patients, cg07016288 was hypomethylated in subjects with an index suicide attempt (IA) in youth ($p < 0.05$) – a characteristic previously associated with greater contribution of violent SA methods and overall lethality(McKean et al., 2018). Moreover, aggregate analyses of open-access data extracted from the prefrontal cortex of suicide completers and non-psychiatric controls demonstrated borderline significant hypermethylation of the same locus ($p = 0.0503$) – apparently driven by males ($p < 0.01$) but not females. These findings indicate gender dependent effects of *CYP2D6*-coupled epigenetic markers in relation to severe suicide attempters and suicide. *CYP2D6*-polymorphisms have been implicated in death by suicide(Wang et al., 2009), findings that were not replicated in a previous larger European study of non-suicide completers subjects exhibiting suicidal behavior(Stephens and De Leon, 2016). This is the first study to implicate epigenetic involvement of *CYP2D6* in predicting severe suicidal behavior and death by suicide, indicating a clinical potential of this

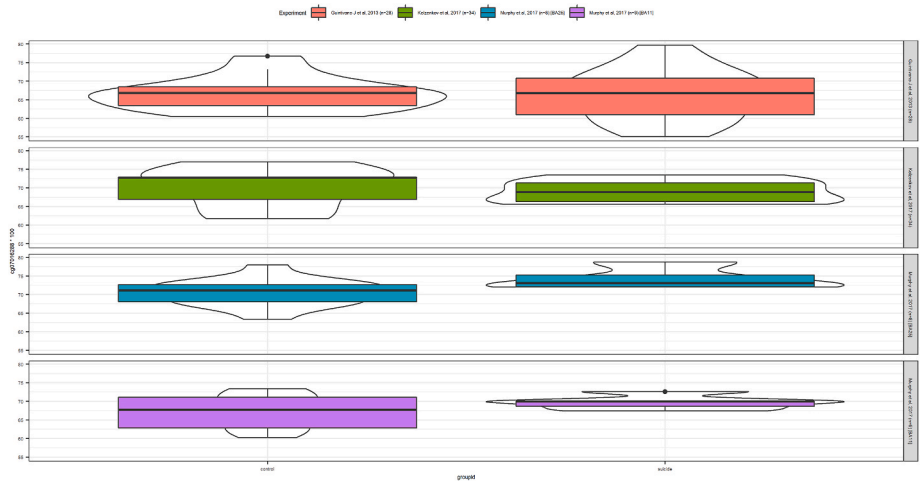

Fig. 2. Boxplots contrasting cg07016288 methylation values by cause of death across the different data sets investigated.

gene.

Strengths of the study include the representative patient population of suicide attempters with thorough diagnostics of the psychiatric disorders and a careful assessment of severity of suicidal behavior(J. Jokinen et al., 2017). Also, possible confounders such as gender, psychotropic medication usage, childhood adversity and comorbidity patterns were carefully considered. It is clear that suicide attempts are highly heterogeneous and prospective studies demonstrate that high intent to die and choice of suicide method evince a higher risk of death by suicide(M. & J., 2015; Stefansson et al., 2012)(J. Jokinen et al., 2017). It can thus be argued that additional knowledge can be gained from results of well-characterized cohorts of suicide attempters. Moreover, the study pertained only to DNA methylation markers measured in whole blood deemed more conciliable with variations occurring in disease-relevant tissue (i.e., brain) and the differential methylation status of the candidate site was indicated also in brain-derived DNA methylation signatures.

5. Limitations

Our study is burdened by several limitations. First, given the relatively small sample size, the main analysis would be underpowered to comprehensively detect subtle changes in DNA methylation. Yet, lower powered studies are appropriate in detecting group-differences with larger effect size, lending further support for the global relevance of the presented findings. Second, as derived methylation changes were small (i.e., mean absolute difference in methylation beta-value of 2.4%), the biological relevance of such subtle changes in a single CpG-site could be questioned. Recent studies, however, implicate that methylation changes in the 1–5% range is associated with extensive transcriptional and translational consequences – a magnitude of alteration deemed particularly relevant in the pathophysiology of complex multifactorial psychiatric conditions(Leenen et al., 2016). Third, similarly to previous epigenetic findings derived from this cohort(J. Jokinen et al., 2017), the detected loci exhibited near-significant hypermethylation in brain tissue of suicide completers (putatively driven by males) whereas demonstrating hypomethylation in whole blood of female severe suicide attempters. The interpretation of these findings is a challenge since making direct comparisons between suicide attempters and suicide completers or blood and brain tissue regarding functional consequences of subtle DNA methylation changes requires complementary preclinical research. Recognized sex differences in suicide-related outcomes(Ho et al., 2021) would indicate sex-specific effects of cg07016288 methylation levels in relation to suicidal severity/death by suicide. Fourth, given the limited scope of the study objectives – to investigate blood-derived DNA methylation markers in relation to severity of suicide attempt – genotyping of *CYP2D6* was not performed. Future studies investigating associations between *CYP2D6* polymorphisms and cg07016288 methylation levels are needed to fully elucidate whether putative underlying pathophysiological mechanisms are conferred by epigenetic, genetic, or combined effects thereof – while simultaneously exploring any potential adjunctive value of *CYP2D6*-coupled epigenetic-genetic composite risk-scores to aid in clinical suicide assessments. Fifth, associations between cg07016288 methylation levels and gene expression levels were not investigated. This was a conscious choice of the authors as the putative prevalence of driving *CYP2D6* gene mutations would need to be accounted for the adequate interpretation of such analyses(Ciuculete et al., 2017). Sixth, CYP2D6 is involved in the metabolism of many medications. Therefore, while psychotropic medication usage was carefully considered, it is possible that non-psychiatric medications also could influence *CYP2D6* activity, putatively exerting downstream effects on DNA methylation at the identified CpG-site. The inability to control such factors – due to data availability issues – could thus be perceived as a limitation. Seventh, the prevalence of non-suicidal self-injury (NSSI) was not included as a covariate in the analysis. However, NSSI was highly prevalent in both the high- and low-risk groups and evenly balanced between the two groups. This should be taken into consideration when interpreting the results of the study. Lastly, exposure to violent behavior can potentially be an effect modifier on severity of suicidal behavior. The KIVS scale suggested no between-group differences regarding exposure to violence in the suicidal low- and high-risk groups. Further studies are needed to investigate the potentially moderating effects of this variable on the association between cg07016288 methylation levels and severity of suicidal behavior.

In conclusion, in an unbiased assessment of 12,930 whole-blood derived methylation probes more conciliable with brain-derived variations, this is the first study to implicate potentially gender-specific effects of a *CYP2D6*-coupled epigenetic marker in predicting age at onset of first suicide attempt and severe suicidal behavior in females and putatively death by suicide in males, indicating a clinical potential of this gene. Ample research previously implicated *CYP2D6* gene variations in relation to suicide deaths(Wang et al., 2009), arguably providing strong support to the relevance of the presented findings (discovered in a non-targeted analysis of 12,930 methylation sites). Future studies investigating associations between *CYP2D6* polymorphisms and cg07016288 methylation levels are needed to fully elucidate whether major underlying pathophysiological mechanisms are conferred by epigenetic, genetic, or combined effects. Results of such studies hold potential to aid efforts aimed at preventing suicide by granting

complementary objective markers that account for both baseline genetic elements (gene polymorphisms; constituting hereditary risk factors that are constant over time) and more variable epigenetic susceptibility factors (DNA methylation; epigenetic mechanisms that in dependency of hereditary and environmental factors change over time) – consistent with a stress and vulnerability model for suicidal risk(Lopez-Castroman et al., 2014).

Data availability

The data underlying the findings presented in this study are available upon reasonable request.

Author contributions

Design of study (A.D.B., H.B.S. J.J., M.Å.). Collection of data (J.J., M. Å., A.D.B, D.M.M, L.K, H.B.S). Data analysis (A.D.B., J.J., E.J., L.K, H.B. S). Drafting of manuscript (A.D.B., E.J.,J.J.) (J.J., M.Å., L.K, H.B.S). contributed to extensive discussions and critical manuscript reading. All authors contributed to and have approved of the final manuscript. All authors are accountable for all aspects of the work.

Role of funding source

Funding for this study was provided by the Swedish Research Council (Project numbers: 5454 (MÅ); K2009–61P-21304-04-4 (J.J); K2009-61X-21305-01-1 (J.J)) and through a regional agreement between Umeå University and Västerbotten County Council (ALF)(J.J.) and by grants provided by the Stockholm County Council (ALF)(J.J.). HBS received funding from the Swedish Brain Research Foundation.

Correspondence

All requests for materials should be addressed directly to Adrian Desai Emmanuel Boström.

Declaration of competing interest

Jussi Jokinen has participated in Advisory Board of Janssen concerning esketamine for MDD with current suicidal ideation. The authors have no other competing interests to declare.

Acknowledgements

Methylation profiling was performed by the SNP&SEQ Technology Platform in Uppsala.

(www.genotyping.se). The facility is part of the National Genomics Infrastructure (NGI) Sweden and.

Science for Life Laboratory. The SNP&SEQ Platform is also supported by the Swedish Research Council.

and the Knut and Alice Wallenberg Foundation. DNA extraction was performed by Latvian BMC, funded

by the Latvian Council of Science European Social Fund and European Regional Development Fund.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2023.02.025.

References

Armstrong, R.A., 2014. When to use the Bonferroni correction. Ophthalmic Physiol. Opt. : The Journal of the British College of Ophthalmic Opticians (Optometrists) 34 (5), 502–508. https://doi.org/10.1111/OPO.12131.

Aryee, M.J., Jaffe, A.E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A.P., Hansen, K.D., Irizarry, R.A., 2014. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 30 (10), 1363–1369. https://doi.org/10.1093/bioinformatics/btu049.

Bani-Fatemi, A., Adanty, C., Dai, N., Dada, O., Strauss, J., Zai, C., Gerretsen, P., Graff, A., De Luca, V., 2020. Genome-wide methylation association with current suicidal ideation in schizophrenia. Journal of Neural Transmission (Vienna, Austria : 1996 127 (9), 1315–1322. https://doi.org/10.1007/S00702-020-02225-8.

Bani-Fatemi, A., Raymond, R., Adanty, C., Dai, N., Gerretsen, P., Graff, A., Nobrega, J.N., De Luca, V., 2021. Global DNA methylation in suicidal ideation and suicide attempt in schizophrenia. Psychiatr. Genet. 65–71. https://doi.org/10.1097/YPG.0000000000000273.

Ciuculete, D.M., Boström, A.E., Voisin, S., Philipps, H., Titova, O.E., Bandstein, M., Nikontovic, L., Williams, M.J., Mwinyi, J., Schiöth, H.B., 2017. A methylome-wide mQTL analysis reveals associations of methylation sites with GAD1 and HDAC3 SNPs and a general psychiatric risk score. Transl. Psychiatry 7 (1). https://doi.org/10.1038/tp.2016.275.

Du, P., Zhang, X., Huang, C.C., Jafari, N., Kibbe, W.A., Hou, L., Lin, S.M., 2010. Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. BMC Bioinf. https://doi.org/10.1186/1471-2105-11-587.

Freeman, D.J., Wilson, K., Thigpen, J., McGee, R.K., 1974. Assessing intention to die in self-injury behavior. In: Psychological Assessment of Suicidal Risk BT - Psychological Assessment of Suicidal Risk.

Guintivano, J., Aryee, M.J., Kaminsky, Z.A., 2013. A cell epigenotype specific model for the correction of brain cellular heterogeneity bias and its application to age, brain region and major depression. Epigenetics 8 (3), 290. https://doi.org/10.4161/EPI.23924.

Hannon, E., Lunnon, K., Schalkwyk, L., Mill, J., 2015. Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes. Epigenetics 10 (11), 1024–1032. https://doi.org/10.1080/15592294.2015.1100786.

Ho, T.C., Gifuni, A.J., Gotlib, I.H., 2021. Psychobiological risk factors for suicidal thoughts and behaviors in adolescence: a consideration of the role of puberty. Mol. Psychiatr. 27 (1), 606–623. https://doi.org/10.1038/s41380-021-01171-5, 2021 27: 1.

Jokinen, Jussi, Forslund, K., Ahnemark, E., Gustavsson, J.P., Nordström, P., Åsberg, M., 2010a. Karolinska Interpersonal Violence Scale predicts suicide in suicide attempters. J. Clin. Psychiatr. 71 (8), 1025–1032. https://doi.org/10.4088/JCP.09M05944BLU.

Jokinen, Jussi, Nordström, A.L., Nordström, P., 2010b. Cholesterol, CSF 5-HIAA, violence and intent in suicidal men. Psychiatr. Res. 178 (1), 217–219. https://doi.org/10.1016/J.PSYCHRES.2008.07.020.

Jokinen, J., Boström, A.E., Chatzittofis, A., Ciuculete, D.M., Öberg, K.G., Flanagan, J.N., Arver, S., Schiöth, H.B., 2017a. Methylation of HPA axis related genes in men with hypersexual disorder. Psychoneuroendocrinology 80. https://doi.org/10.1016/j.psyneuen.2017.03.007.

Jokinen, J., Boström, A.E., Dadfar, A., Ciuculete, D.M., Chatzittofis, A., Åsberg, M., Schiöth, H.B., 2017b. Epigenetic Changes in the CRH Gene Are Related to Severity of Suicide Attempt and a General Psychiatric Risk Score in Adolescents. EBioMedicine. https://doi.org/10.1016/j.ebiom.2017.12.018.

Jollant, F., Bellivier, F., Leboyer, M., Astruc, B., Torres, S., Verdier, R., Castelnau, D., Malafosse, A., Courtet, P., 2005. Impaired decision making in suicide attempters. Am. J. Psychiatr. https://doi.org/10.1176/appi.ajp.162.2.304.

Kouter, K., Zupanc, T., Videtič Paska, A., 2019. Genome-wide DNA methylation in suicide victims revealing impact on gene expression. J. Affect. Disord. 253, 419–425. https://doi.org/10.1016/J.JAD.2019.04.077.

Kozlenkov, A., Jaffe, A.E., Timashpolsky, A., Apontes, P., Rudchenko, S., Barbu, M., Byne, W., Hurd, Y.L., Horvath, S., Dracheva, S., 2017. DNA methylation profiling of human prefrontal cortex neurons in heroin users shows significant difference between genomic contexts of hyper- and hypomethylation and a younger epigenetic age. Genes 8 (6), 2–15. https://doi.org/10.3390/GENES8060152.

Lê, S., Josse, J., Mazet, F., 2008. Package ' FactoMineR. J. Stat. Software 25 (1), 1–18. https://doi.org/10.1007/978-3-540-74686-7.

Leek, J.T., Johnson, W.E., Parker, H.S., Jaffe, A.E., Storey, J.D., 2012. The SVA package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics. https://doi.org/10.1093/bioinformatics/bts034.

Leenen, F.A.D., Muller, C.P., Turner, J.D., 2016. DNA methylation: conducting the orchestra from exposure to phenotype? Clin. Epigenet. 8 (1), 92. https://doi.org/10.1186/s13148-016-0256-8.

Lopez-Castroman, J., Olié, E., Courtet, P., 2014. Stress and vulnerability: a developing model for suicidal risk. Suicide: Phenomenology and Neurobiology 87–100. https://doi.org/10.1007/978-3-319-09964-4_5/COVER.

Mansell, G., Gorrie-Stone, T.J., Bao, Y., Kumari, M., Schalkwyk, L.S., Mill, J., Hannon, E., 2019. Guidance for DNA methylation studies: statistical insights from the Illumina EPIC array. BMC Genom. https://doi.org/10.1186/s12864-019-5761-7.

McKean, A.J.S., Pabbati, C.P., Geske, J.R., Bostwick, J.M., 2018. Rethinking lethality in youth suicide attempts: first suicide attempt outcomes in youth ages 10 to 24. J. Am. Acad. Child Adolesc. Psychiatry 57 (10), 786–791. https://doi.org/10.1016/J.JAAC.2018.04.021.

Menon, V., Kattimani, S., Sarkar, S., Sathyanarayanan, G., Subramanian, K., Velusamy, S. K., 2018. Age at onset of first suicide attempt: exploring the utility of a potential candidate variable to subgroup attempters. Asian Journal of Psychiatry 37, 40–45. https://doi.org/10.1016/J.AJP.2018.08.006.

Mishara, B.L., Weisstub, D.N., 2021. Genetic testing for suicide risk assessment: theoretical premises, research challenges and ethical concerns. Prev. Med. 152 (Pt 1) https://doi.org/10.1016/J.YPMED.2021.106685.

Morris, T.J., Butcher, L.M., Feber, A., Teschendorff, A.E., Chakravarthy, A.R., Wojdacz, T.K., Beck, S., 2014. ChAMP: 450k chip analysis methylation pipeline. Bioinformatics 30 (3), 428–430. https://doi.org/10.1093/bioinformatics/btt684.

Murphy, T.M., Crawford, B., Dempster, E.L., Hannon, E., Burrage, J., Turecki, G., Kaminsky, Z., Mill, J., 2017a. Methylomic profiling of cortex samples from completed suicide cases implicates a role for PSORS1C3 in major depression and suicide. Transl. Psychiatry 7 (1). https://doi.org/10.1038/TP.2016.249.

Murphy, T.M., Crawford, B., Dempster, E.L., Hannon, E., Burrage, J., Turecki, G., Kaminsky, Z., Mill, J., 2017b. Methylomic profiling of cortex samples from completed suicide cases implicates a role for PSORS1C3 in major depression and suicide. Transl. Psychiatry 7 (1). https://doi.org/10.1038/TP.2016.249.

Nagy, C., Torres-Platas, S.G., Mechawar, N., Turecki, G., 2017. Repression of astrocytic connexins in cortical and subcortical brain regions and prefrontal enrichment of H3K9me3 in depression and suicide. Int. J. Neuropsychopharmacol. 20 (1), 50–57. https://doi.org/10.1093/IJNP/PYW071.

Policicchio, S., Washer, S., Viana, J., Iatrou, A., Burrage, J., Hannon, E., Turecki, G., Kaminsky, Z., Mill, J., Dempster, E.L., Murphy, T.M., 2020a. Genome-wide DNA methylation meta-analysis in the brains of suicide completers. Transl. Psychiatry 10 (1), 1–13. https://doi.org/10.1038/s41398-020-0752-7, 2020 10:1.

Policicchio, S., Washer, S., Viana, J., Iatrou, A., Burrage, J., Hannon, E., Turecki, G., Kaminsky, Z., Mill, J., Dempster, E.L., Murphy, T.M., 2020b. Genome-wide DNA methylation meta-analysis in the brains of suicide completers. Transl. Psychiatry 10 (1), 1–13. https://doi.org/10.1038/s41398-020-0752-7, 2020 10:1.

Policicchio, S., Washer, S., Viana, J., Iatrou, A., Burrage, J., Hannon, E., Turecki, G., Kaminsky, Z., Mill, J., Dempster, E. L., & Murphy, T. M. (n.d.). Genome-wide DNA methylation meta-analysis in the brains of suicide completers. 2020c https://doi.org/10.1038/s41398-020-0752-7.

Price, M.E., Cotton, A.M., Lam, L.L., Farré, P., Emberly, E., Brown, C.J., Robinson, W.P., Kobor, M.S., 2013. Additional annotation enhances potential for biologically-relevant analysis of the Illumina Infinium HumanMethylation450 BeadChip array. Epigenet. Chromatin 6 (1), 4. https://doi.org/10.1186/1756-8935-6-4.

Rask-Andersen, M., Bringeland, N., Nilsson, E.K., Bandstein, M., Búcaro, M.O., Vogel, H., Schürmann, A., Hogenkamp, P.S., Benedict, C., Schiöth, H.B., 2016. Postprandial alterations in whole-blood DNA methylation are mediated by changes in white blood cell composition. Am. J. Clin. Nutr. 104 (2), 518–525. https://doi.org/10.3945/ajcn.115.122366.

Schalkwyk, L.C., Pidsley, R., Wong, C.C.Y., 2013. wateRmelon: Illumina 450 methylation array normalization and metrics. In: R Package, p. 2, version 1.2.

Schneider, E., El Hajj, N., Müller, F., Navarro, B., Haaf, T., 2015. Epigenetic dysregulation in the prefrontal cortex of suicide completers. Cytogenet. Genome Res. 146 (1), 19–27. https://doi.org/10.1159/000435778.

Sinai, C., Hirvikoski, T., Nordströ, A.-L., Nordströ, P., Sa Nilsonne, A. °, Wilczek, A., Sberg, M. A. °, & Jokinen, J. (n.d.). Hypothalamic pituitary thyroid axis and exposure to interpersonal violence in childhood among women with borderline personality disorder. 2014 https://doi.org/10.3402/ejpt.v5.23911.

Sinai, C., Hirvikoski, T., Vansvik, E.D., Nordström, A.L., Linder, J., Nordström, P., Jokinen, J., 2009. Thyroid hormones and personality traits in attempted suicide. Psychoneuroendocrinology 34 (10), 1526–1532. https://doi.org/10.1016/J.PSYNEUEN.2009.05.009.

Sinai, C., Hirvikoski, T., Wiklander, M., Nordström, A.L., Nordström, P., Nilsonne, Å., Wilczek, A., Åsberg, M., Jokinen, J., 2018. Exposure to interpersonal violence and risk of post-traumatic stress disorder among women with borderline personality disorder. Psychiatr. Res. 262, 311–315. https://doi.org/10.1016/J.PSYCHRES.2018.01.047.

Smyth, G.K., 2004. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. In: Statistical Applications in Genetics and Molecular Biology, 3. https://doi.org/10.2202/1544-6115.1027. Article3.

Stefansson, J., Nordström, P., Jokinen, J., 2012. Suicide intent scale in the prediction of suicide. J. Affect. Disord. https://doi.org/10.1016/j.jad.2010.11.016.

Stenbacka, M., Jokinen, J., 2015. Violent and non-violent methods of attempted and completed suicide in Swedish young men: the role of early risk factors. BMC Psychiatr. 15 (1) https://doi.org/10.1186/S12888-015-0570-2.

Stephens, D.B., De Leon, J., 2016. CYP2D6 Ultra-rapid Metabolizer Phenotype Not Associated with Attempted Suicide in a Large Sample of Psychiatric Inpatients, 17, pp. 1295–1304. https://doi.org/10.2217/PGS-2016-0086. *Dx.Doi.Org/10.2217/Pgs-2016-0086*, 12.

Träskman, L., Åsberg, M., Bertilsson, L., Sjüstrand, L., 1981. Monoamine Metabolites in CSF and Suicidal Behavior. Archives of General Psychiatry. https://doi.org/10.1001/archpsyc.1981.01780310031002.

van Iterson, M., van Zwet, E.W., Heijmans, B.T., 't Hoen, P.A.C., van Meurs, J., Jansen, R., Franke, L., Boomsma, D.I., Pool, R., van Dongen, J., Hottenga, J.J., van Greevenbroek, M.M.J., Stehouwer, C.D.A., van der Kallen, C.J.H., Schalkwijk, C.G., Wijmenga, C., Zhernakova, S., Tigchelaar, E.F., Eline Slagboom, P., et al., 2017. Controlling bias and inflation in epigenome- and transcriptome-wide association studies using the empirical null distribution. Genome Biol. https://doi.org/10.1186/s13059-016-1131-9.

Viana, J., Hannon, E., Dempster, E., Pidsley, R., Macdonald, R., Knox, O., Spiers, H., Troakes, C., Al-Saraj, S., Turecki, G., Schalkwyk, L.C., Mill, J., 2017. Schizophrenia-associated methylomic variation: molecular signatures of disease and polygenic risk burden across multiple brain regions. Hum. Mol. Genet. 26 (1), 210–225. https://doi.org/10.1093/HMG/DDW373.

Wagner, J.R., Busche, S., Ge, B., Kwan, T., Pastinen, T., Blanchette, M., 2014. The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. Genome Biol. 15 (2), R37. https://doi.org/10.1186/gb-2014-15-2-r37.

Wang, B., Yang, L.P., Zhang, X.Z., Huang, S.Q., Bartlam, M., Zhou, S.F., 2009. New Insights into the Structural Characteristics and Functional Relevance of the Human Cytochrome P450 2D6 Enzyme, 41, pp. 573–643. https://doi.org/10.1080/03602530903118729. *Dx.Doi.Org/10.1080/03602530903118729*, 4.

World Health Organization (WHO), 2021. Suicide Worldwide in 2019: Global Health Estimates, 35. https://www.who.int/publications/i/item/9789240026643.

Zaboli, G., Gizatullin, R., Nilsonne, Å., Wilczek, A., Jönsson, E.G., Ahnemark, E., Åsberg, M., Leopardi, R., 2006. Tryptophan hydroxylase-1 gene variants associate with a group of suicidal borderline women. Neuropsychopharmacology 31 (9), 1982–1990. https://doi.org/10.1038/sj.npp.1301046, 2006 31:9.